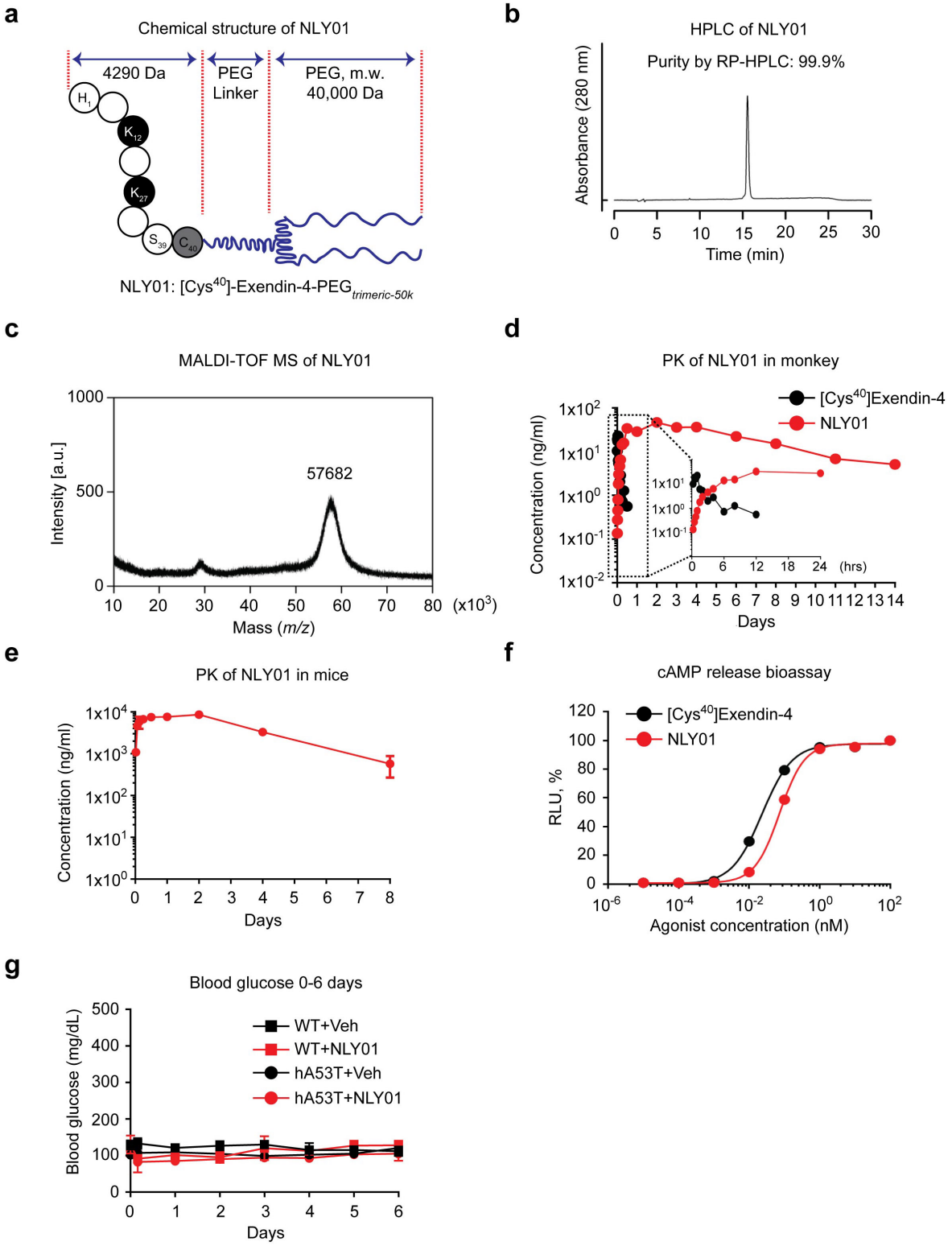
Supplementary Information

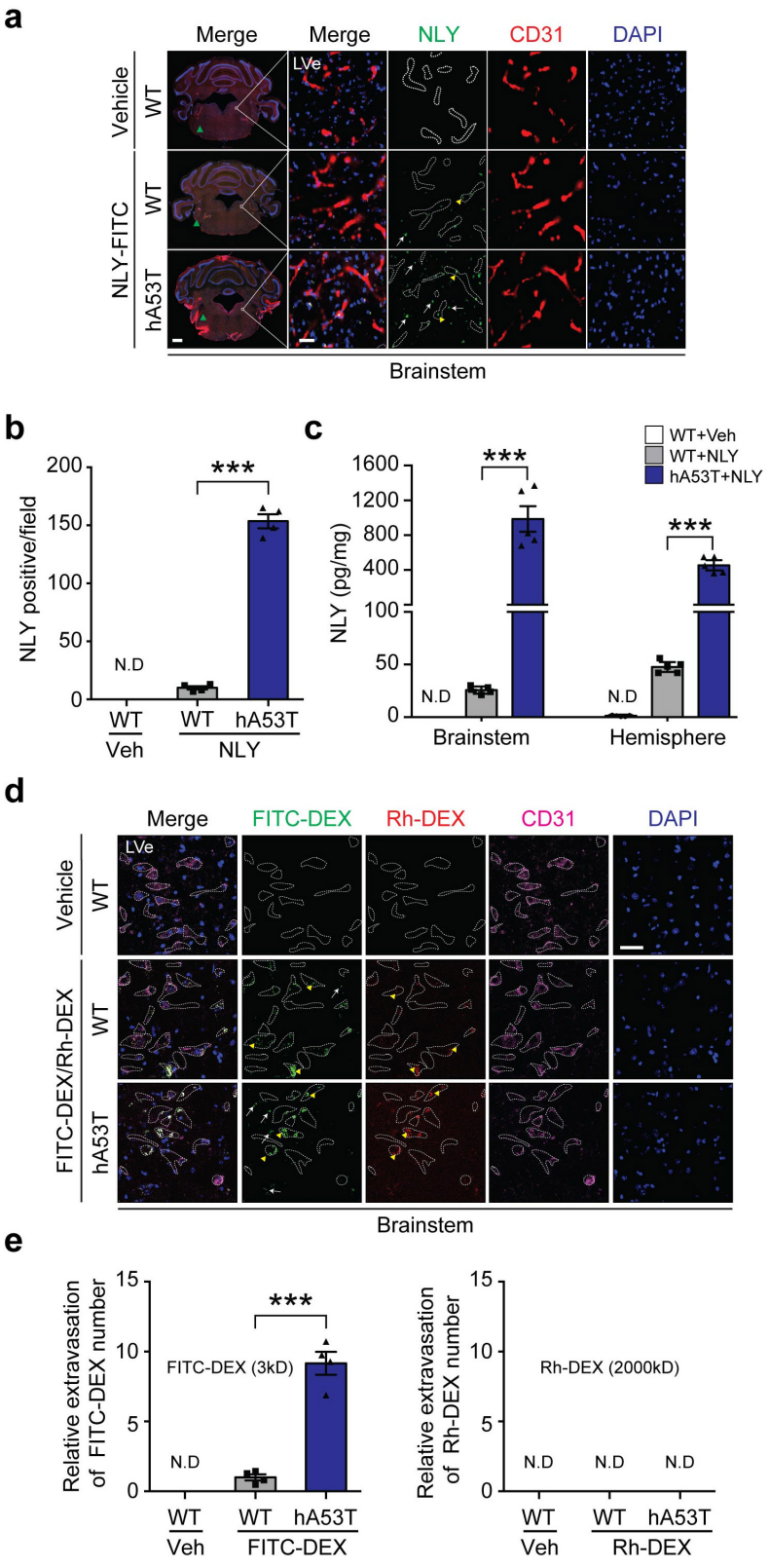
Supplementary Figure 1



Supplementary Figure 1. Structure and characterization of NLY01. (**a**) Structure of NLY01. NLY01 is PEGylated [Cys^{40}]Exendin-4. (**b**) Representative RP-HPLC chromatogram of NLY01.

Purity > 99.9%. Each analysis was independently repeated three times. (**c**) Representative MALDI-TOF mass spectrum of NLY01. Molecular weight: 57,682 Da. The evaluation was independently repeated three times. (**d**) Pharmacokinetic (PK) profile of subcutaneously administered [Cys^{40}]Exendin-4 and NLY01 (32 μg/kg) in cynomolgus monkeys. Data are expressed as the mean concentrations of peptide residues (n=2, biologically independent animals). PK profiles are summarized in Supplementary Table 1. (**e**) PK profile of subcutaneously administered NLY01 (1 mg/kg) in mice. Data are expressed as the mean concentrations of peptide residues ± S.E.M (n=3 at each time point, biologically independent animals). (**f**) Biological activity of [Cys^{40}]Exendin-4 and NLY01 was analyzed by cAMP production stimulated by [Cys^{40}]Exendin-4 or NLY01 in GLP-1R transfected HEK-293 cells (HEK-293/CRE-LUC/GLP1R cells). Data represent the mean of triplicates for one representative experiment that was repeated three independent times. EC_{50}: [Cys^{40}]Exendin-4, 0.023 nM; NLY01, 0.073 nM. (**g**) Blood glucose changes of 10-month-old WT and hA53T α-syn Tg mice subcutaneously administered with PBS or NLY01 (3 mg/kg) on day 0 and day 3. Data are expressed as the mean blood glucose (mg/dL) concentrations ± S.E.M (n=3, biologically independent animals).

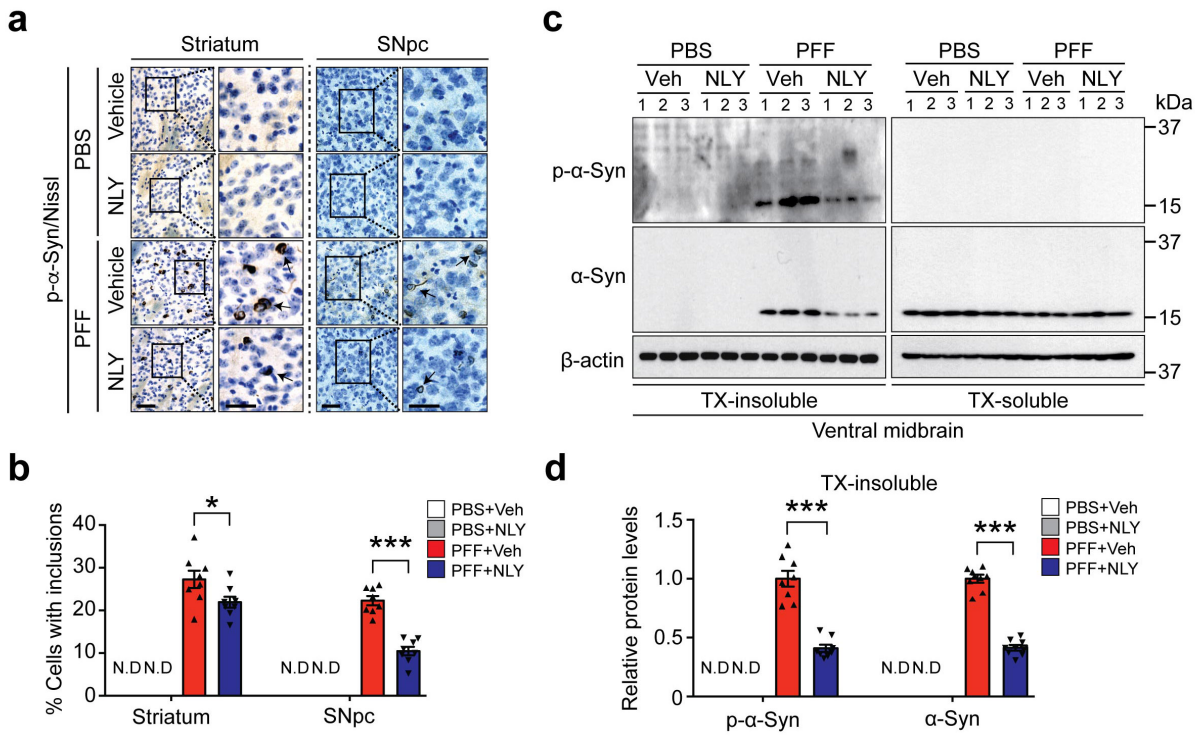
Supplementary Figure 2



Supplementary Figure 2. Accumulation of NLY01 in brain. (**a**) 10-month-old WT and hA53T α-syn Tg mice were treated with vehicle or NLY01-FITC (3 mg/kg) for 7 days. Localization of

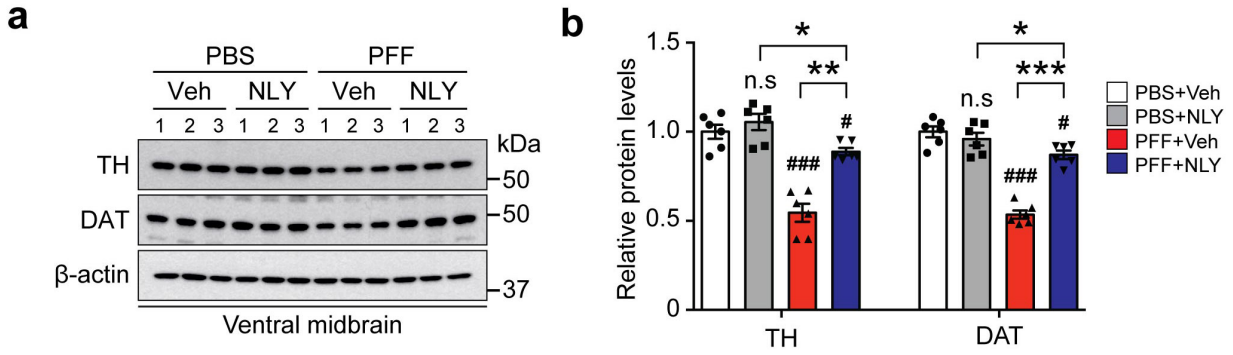
NLY01 (green) was assessed by a confocal microscopy. CD31 (red) served as a vessel marker. Scale bar, 500 μm or 10 μm. Green arrow head indicates a tile image line, yellow arrow head indicates a vessel, and white arrow indicates NLY01. (**b**) Quantification of NLY01 dot number in brainstem. Error bars represent the mean ± S.E.M. (n=4, biologically independent animals, p value < 0.0001). (**c**) 10-month-old WT and hA53T α-syn Tg mice were treated with vehicle or NLY01 (3 mg/kg) for 7 days. NLY01 concentration was quantified using ELISA. Error bars represent the mean ± S.E.M. (n=5, biologically independent animals, p value < 0.0001). (**d**) 10-month-old WT and hA53T α-syn Tg mice were intravenously injected with FITC-Dextran (3 kD), and Rhodamine (Rh)-Dextran (2000 kD) 1 hr before sacrifice. Localization of Dextran-FITC (green dot) and Rh-Dextran (red dot) was assessed by confocal microscopy. CD31 (violet) served as a vessel marker. (n=4, biologically independent animals). Scale bar, 10 μm. Yellow arrow head indicates a vessel, and white arrow indicates Dextran. (**e**) Quantification of FITC-Dextran (green dot) and Rh-Dextran number in brainstem. Error bars represent the mean ± S.E.M. (n=4, biologically independent animals, p value < 0.0001). Unpaired two-tailed Student's t test for statistical significance. ***$P < 0.001$ vs. WT with NLY or WT with FITC-DEX. ND, not detected.

Supplementary Figure 3



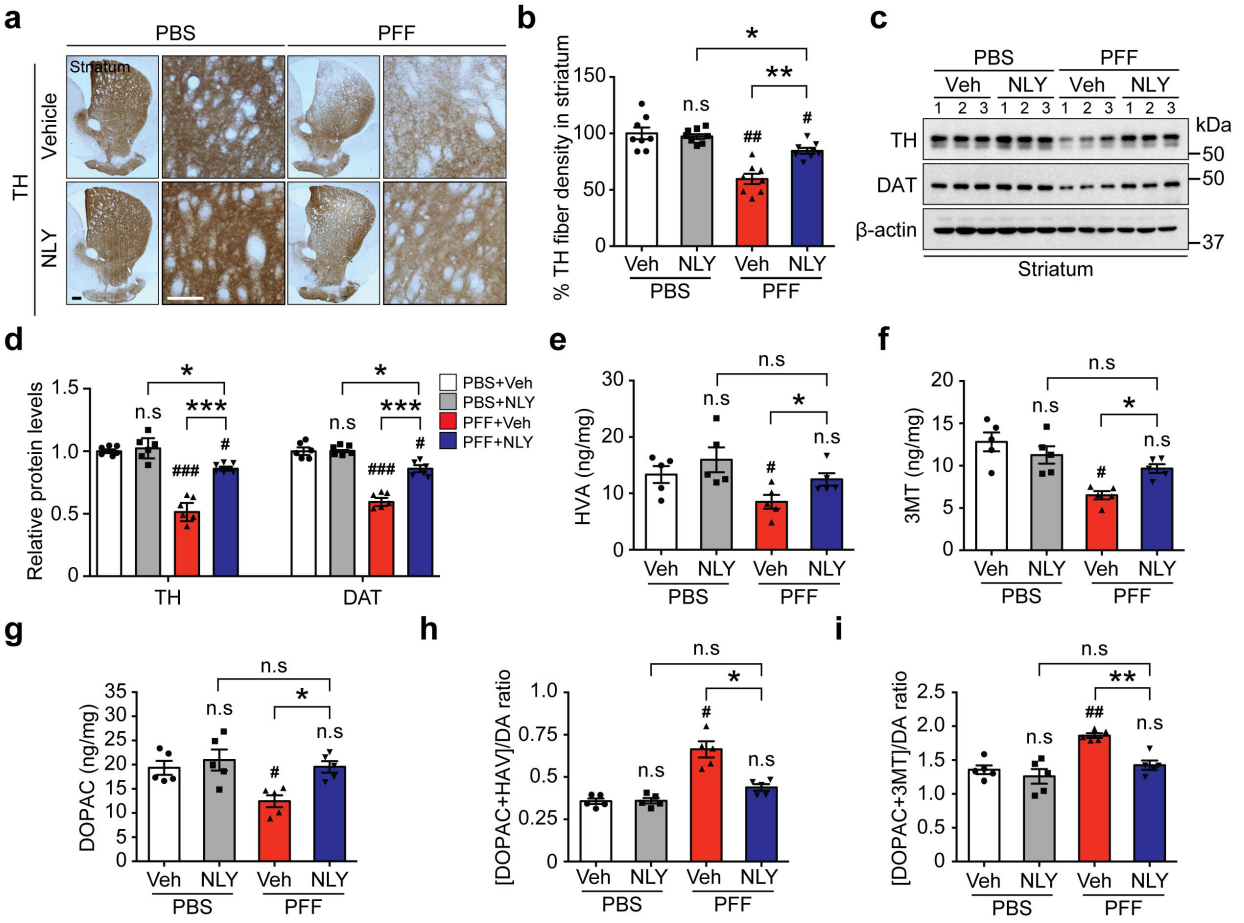
Supplementary Figure 3. NLY01 protects against α-syn PFF-induced LB-like pathology. (**a**) Representative immunohistochemistry for p-α-syn^{ser129} (p-α-syn) in the striatum and SNpc of the ventral midbrain (n=8, biologically independent animals). Scale bar, 50 μm or 25 μm. (**b**) Quantification of the striatum and SNpc neurons with p-α-syn^{ser129} positive inclusions. Error bars represent the mean ± S.E.M. (n=8, biologically independent animals, Striatum, p value = 0.042 or SNpc, p value < 0.0001). Unpaired two-tailed Student's t test for statistical significance. (**c**) Representative immunoblots of α-syn, p-α-syn^{ser129}, and β-actin in the detergent-insoluble fraction and detergent-soluble fraction of the ventral midbrain (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots, n=8, biologically independent animals) (**d**) Quantification of α-syn and p-α-syn^{ser129} protein levels in the detergent-insoluble (TX-100) fraction normalized to β-actin. Error bars represent the mean ± S.E.M. (n=8, biologically independent animals, p value < 0.0001). Unpaired two-tailed Student's t test for statistical significance. *P < 0.05, ***P < 0.001 vs. α-syn PFF stereotaxic injected mice with vehicle. N.D, not detection.

Supplementary Figure 4



Supplementary Figure 4. NLY01 rescues PFF reduction in TH and DAT levels in the ventral midbrain. (**a**) Representative immunoblots of TH, DAT, and β-actin in the ventral midbrain (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots, n=6, biologically independent animals). (**b**) Quantification of TH, and DAT protein levels normalized to β-actin. Error bars represent the mean ± S.E.M. (n=6, biologically independent animals). Two-way ANOVA was used to test for statistical significance, followed by Tukey's multiple comparisons test. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. PBS stereotaxic injected mice with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF stereotaxic injected mice with NLY01. n.s, not significant.

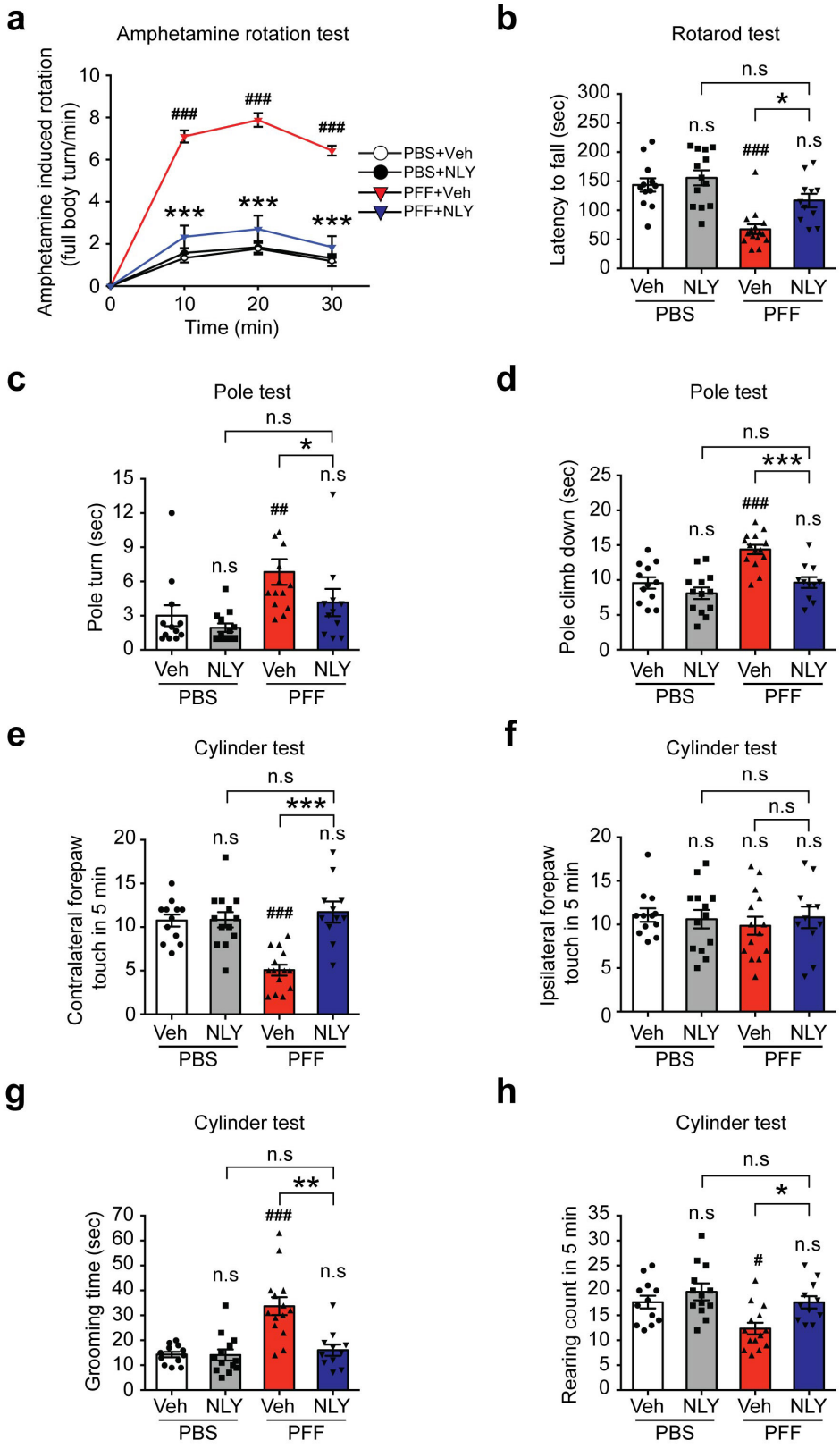
Supplementary Figure 5



Supplementary Figure 5. NLY01 rescues PFF-induced dopaminergic terminal loss and depletion of dopamine metabolites in the striatum. (**a**) Representative photomicrograph of striatal sections stained for TH immunoreactivity. High power view of TH fiber density in the striatum. (n=8, biologically independent animals). Scale bar, 100 µm or 50 µm. **(b)** Quantification of dopaminergic fiber densities in the striatum using Image J software (NIH). Error bars represent the mean ± S.E.M. (n=8, biologically independent animals). **(c)** Representative immunoblots of TH, DAT, and β-actin in the striatum of PBS and α-syn PFF stereotaxic injected mice treated with vehicle or NLY01 (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots, n=6, biologically independent animals). **(d)** Quantification of TH, and DAT levels in the striatum normalized to β-actin. Error bars represent the mean ± S.E.M. (n=6, biologically independent animals). **(e-i)** Striatal levels of DA metabolites were measured by HPLC-ECD. The levels of **(e)** HVA, **(f)** 3MT, and, **(g)** DOPAC were measured in the striatum from PBS and α-syn PFF stereotaxic injected mice treated with vehicle or NLY01. **(h)** DA turnover [(DOPAC+HVA/DA)] and **(i)** [(DOPAC+3MT)/DA] were calculated from the striatum. Error bars represent the mean ± S.E.M. (n=5, biologically independent animals). Two-way ANOVA was used

to test for statistical significance, followed by Tukey's multiple comparisons test. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS stereotaxic injected mice with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF stereotaxic injected mice with NLY01. n.s, not significant.

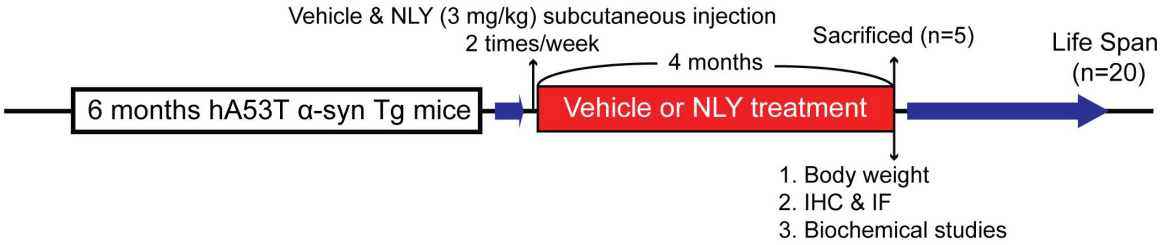
Supplementary Figure 6



Supplementary Figure 6. NLY01 protects against α-syn PFF-induced motor defects. 6 months after PBS or α-syn PFF stereotaxic brain injections, behavioral tests were performed in vehicle or

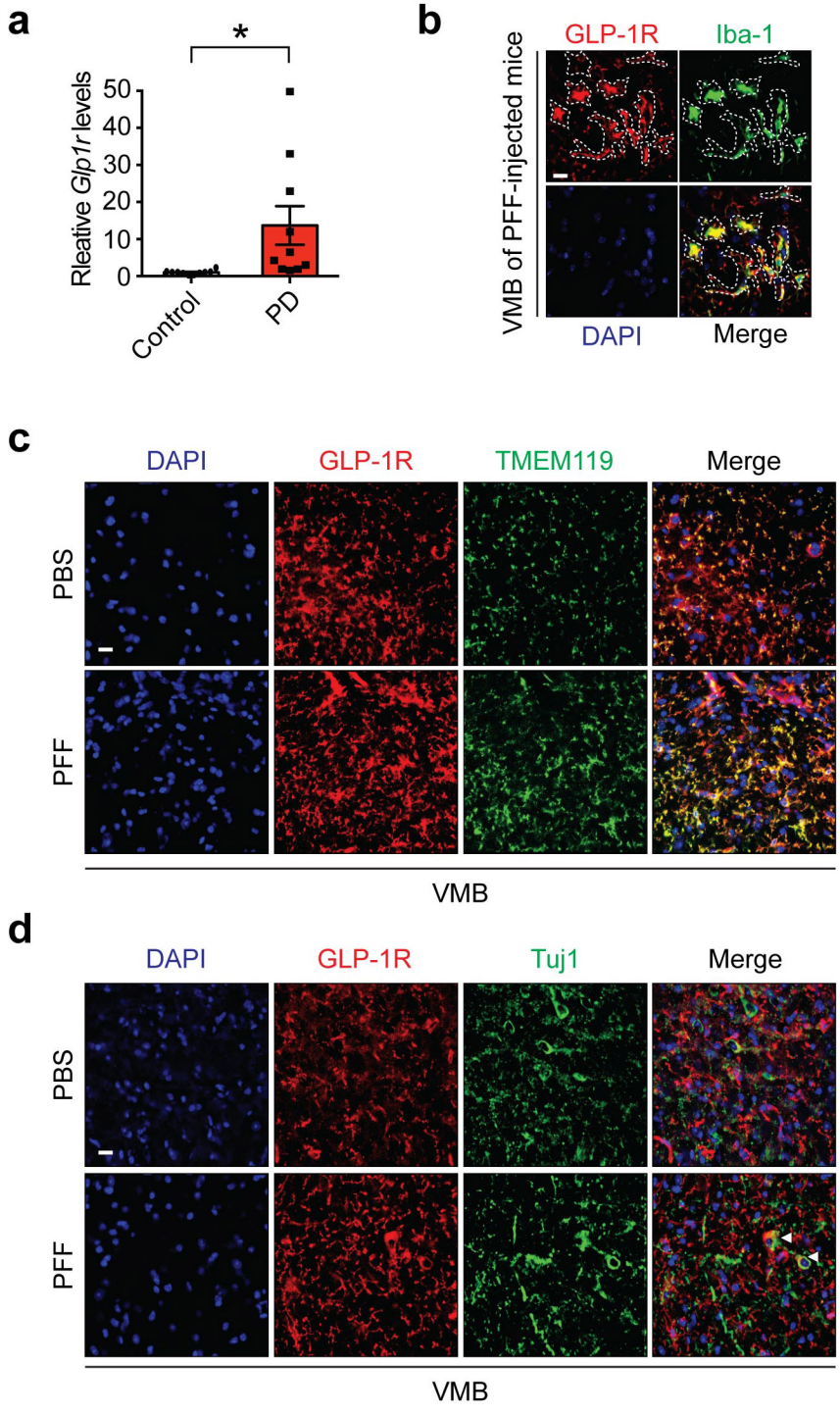
NLY01 treated mice. Behavioral abnormalities were improved in mice treated with NLY01. Results of animals on the (**a**) amphetamine rotation test, **(b)** rotarod, (**c**, **d**) pole test, and (**e-h**) cylinder test. Error bars represent the mean ± S.E.M. (n=12 PBS stereotaxic injection with vehicle, n=13 PBS stereotaxic injection with NLY01, n=14 α-syn PFF stereotaxic injection with vehicle, and n=11 α-syn PFF stereotaxic injection with NLY01, biologically independent animals). Two-way ANOVA was used for statistical analysis followed by Tukey's multiple comparisons test for multiple group comparisons. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS stereotaxic injected mice with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF stereotaxic injected mice with NLY01. Maximum time to climb down the pole was limited to 60 sec. n.s, not significant.

Supplementary Figure 7



Supplementary Figure 7. Scheme of NLY01 treatment of human A53T α-synuclein transgenic (hA53T α-syn Tg) mice. 6-month-old WT and hA53T α-syn Tg mice were treated with vehicle or NLY01 for 4 months or until moribund. 4 months after NLY01 treatment, mice were sacrificed. Animal numbers used for immunohistochemistry (n=5) and biochemical studies (n=5) are indicated.

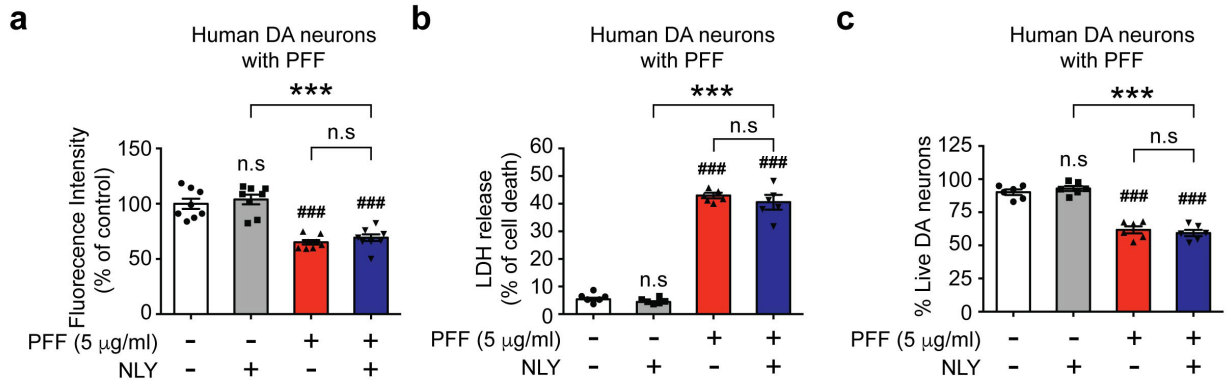
Supplementary Figure 8



Supplementary Figure 8. Expression of GLP-1R. (a) *Glp1r* mRNA expression was increased in the substantia nigra region of brains affected by PD (n=10, biologically independent human postmortem brain), as compared to controls (n=10, biologically independent human postmortem brain). The fold induction level of *Glp1r* mRNA was expressed as the mean ± S.E.M., Unpaired two-tailed Student's t test, p value = 0.0257. (**b**) Co-localization of GLP1-R (red) and Iba-1 (green) in VMB of α-syn PFF injected mice (n=4, biologically independent animals). Scale bar, 10 µm. (**c**)

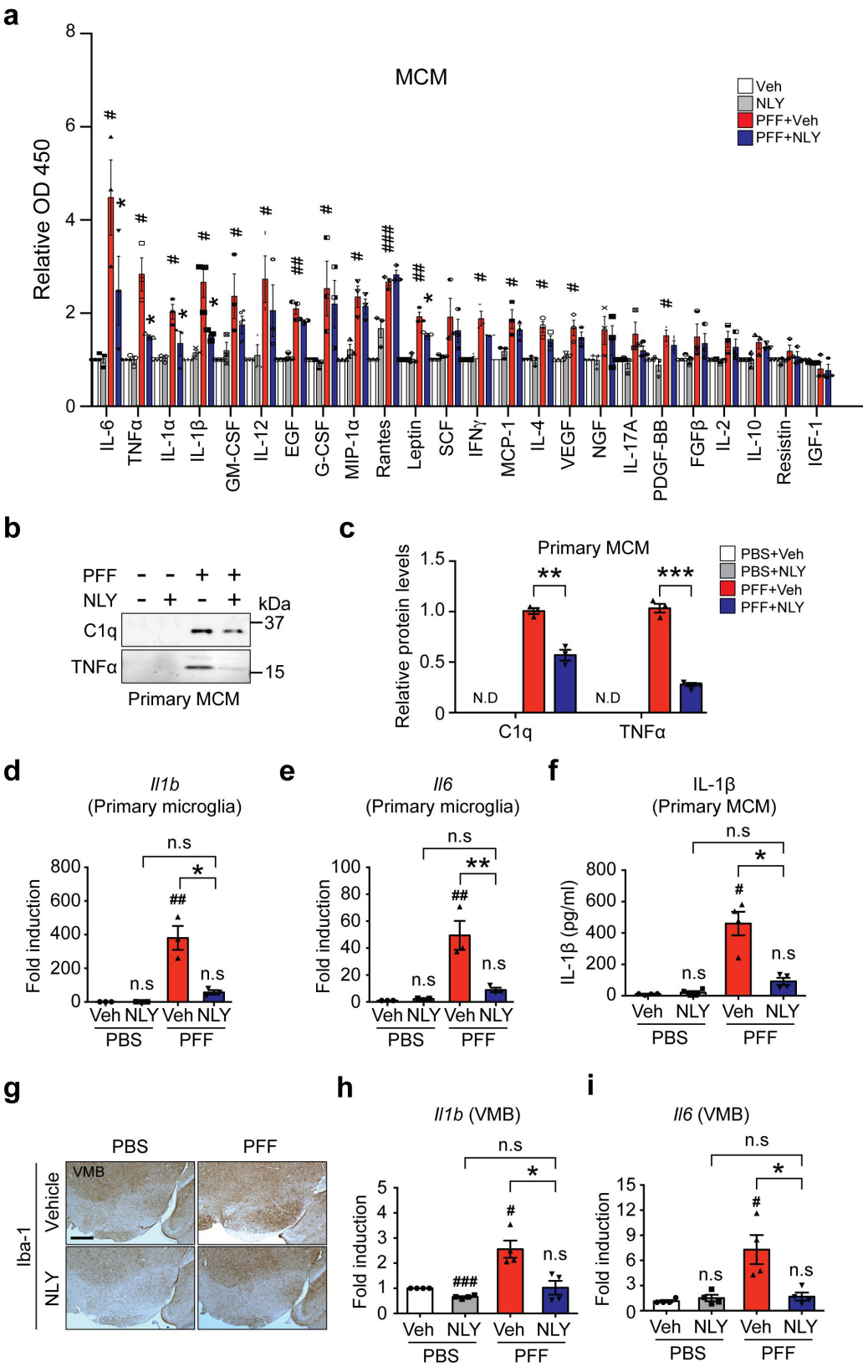
Co-localization of GLP1-R (red) and TMEM119 (green) in VMB of α-syn PFF injected mice (n=4, biologically independent animals). Scale bar, 20 μm. (**d**) Co-localization of GLP1-R (red) and Tuj1 (green) in VMB of α-syn PFF injected mice. White arrow head indicates a co-localization of GLP1-R (red) and Tuj1 (n=4, biologically independent animals). Scale bar, 20 μm.

Supplementary Figure 9



Supplementary Figure 9. NLY01 fails to protect human dopamine neurons (DA) from α-syn PFF toxicity. DIV 60 human dopaminergic (DA) neurons differentiated from H9 ESCs were pretreated with NLY01 (1 μM) followed by administration of human α-syn PFF (5 μg/ml). Human DA neuronal cell death was determined by the (**a**) alamarBlue assay (n=8, biologically independent human dopaminergic neurons), (**b**) LDH assay (n=6, biologically independent human dopaminergic neurons), and (**c**) Trypan Blue live cell counting (n=6, biologically independent human dopaminergic neurons). Bars represent the mean ± S.E.M. Two-way ANOVA was used for statistical analysis followed by Tukey's multiple comparisons test for multiple group comparisons. $^{###}P < 0.001$ vs. PBS only; $^{***}P < 0.001$ vs. α-syn PFF with NLY01. n.s, not significant.

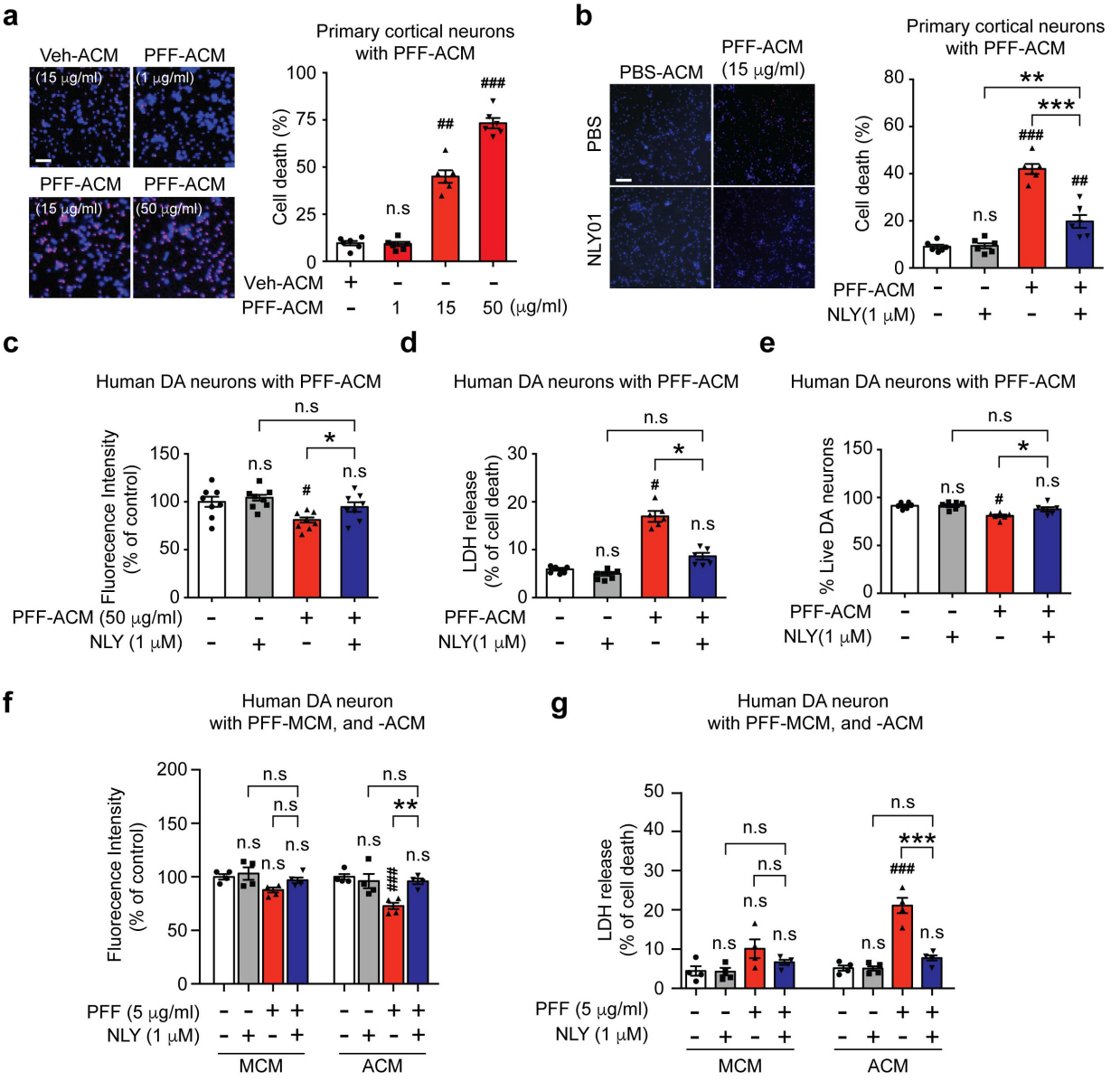
Supplementary Figure 10



Supplementary Figure 10. Microglial function of NLY01. (a) Cytokine array screening of α-syn PFF-activated MCM. Primary microglia were pretreated with PBS or NLY01 (1 μM) for 30

min, and then further incubated with α-syn PFF (1 μg/ml) for 18 hrs. The levels of each cytokine were screened by ELISA-based cytokine arrays. Bars indicate mean ± S.E.M. (n=3, biologically independent primary microglia conditional media). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS alone; $^{*}P < 0.05$ vs. α-syn PFF. (**b**) Representative western blot (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots) and (**c**) quantification of C1q and TNFα proteins in MCM. Pretreatment of NLY01 (1 μM) prevented the secretion of C1q and TNFα proteins. Bars represent the mean ± S.E.M. (n=3, biologically independent primary microglia conditional media, C1q, p value = 0.002 and TNFα, p value < 0.0001). Unpaired two-tailed Student's t test for statistical significance. (**d**-**e**) Quantitative PCR analysis of NLY01 (1 μM) pretreatment on α-syn PFF-activated (1 μg/ml) microglia, (**d**) *Il1b,* and (**e**) *Il6*. Bars represent the mean ± S.E.M (n=3, biologically independent primary microglia). (**f**) Cytokine analysis of IL-1β in MCM 18 hrs after α-syn PFF treatment by ELISA. Bars represent the mean ± S.E.M. (n=4, biologically independent primary microglia conditional media). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. PBS with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. α-syn PFF with NLY01. (**g**) Representative immunohistochemical images of Iba-1 in ventral midbrain (n=6, biologically independent animals). Scale bar, 500 μm. (**h, i**) Quantitative PCR of (**h**) *Il1b*, and (**i**) *Il6* in the ventral midbrain of α-syn PFF injected mice. Bars represent the mean ± S.E.M. (n=4, biologically independent animals). Two-way ANOVA was used for statistical analysis followed by Tukey's multiple comparisons test for multiple group comparisons. $^{\#}P < 0.05$ vs. PBS stereotaxic injected mice with vehicle; $^{*}P < 0.05$ vs. or α-syn PFF stereotaxic injected mice with NLY01. n.s, not significant; MCM, microglial conditioned media.

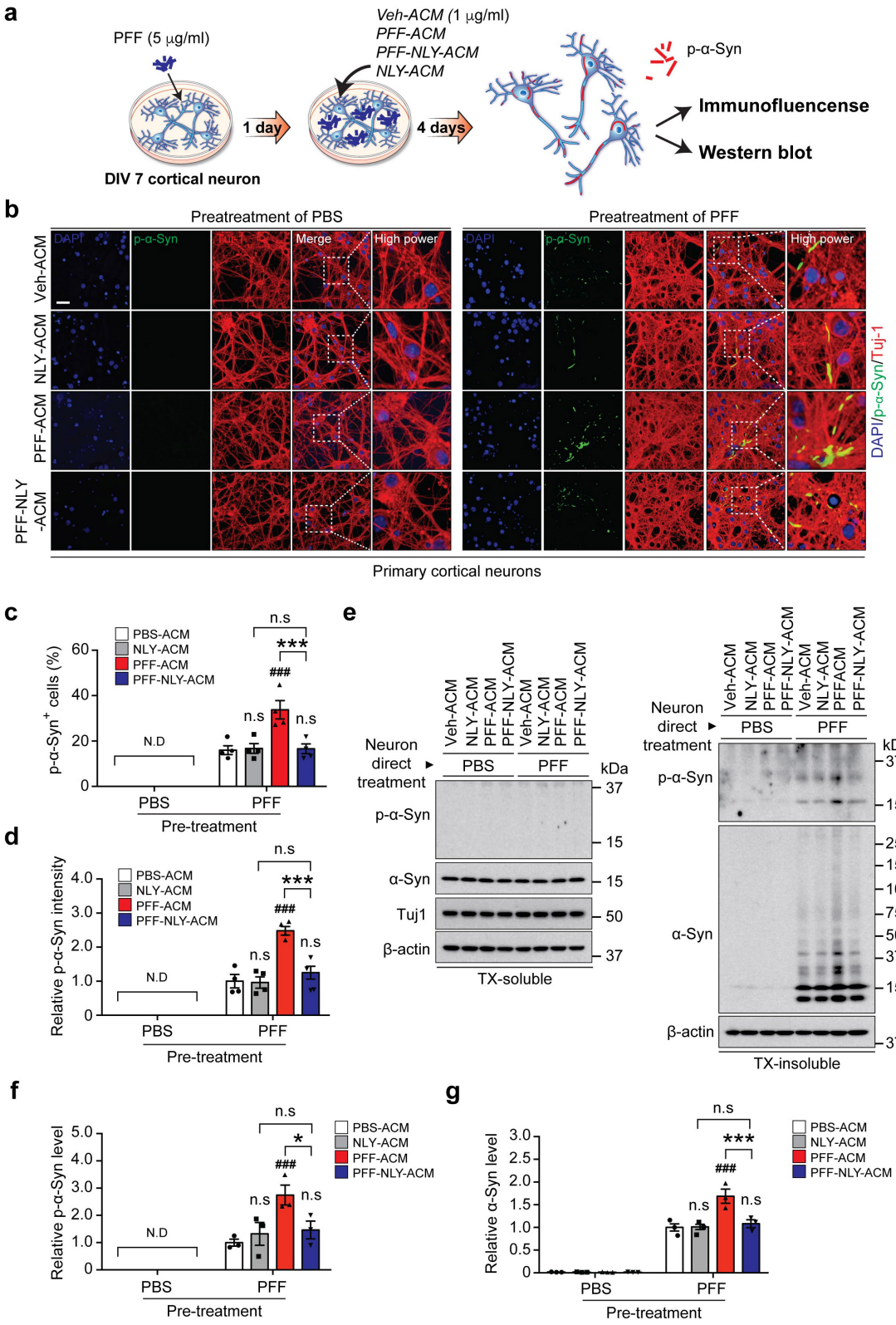
Supplementary Figure 11



Supplementary Figure 11. Inhibition of α-syn PFF-mediated astrocyte conditional media (ACM)-induced neuronal death by NLY01. (**a**) Hoechst and propidium iodide (PI) staining representative images showing death of mouse primary cortical neurons by α-syn PFF-ACM in a dose-dependent manner (left, Propidium iodide stain in red indicates dead cells). Quantification of cell death at 48 hrs after α-syn PFF-ACM treatment. Bars indicate mean ± S.E.M. (n=6, biologically independent primary cortical neurons). Scale bar, 10 μm. (**b**) NLY01 prevents α-syn PFF-ACM toxicity as assessed by Hoechst and PI staining. Mouse primary cortical neurons were incubated with α-syn PFF-ACM with or without NLY01 (1 μM). The toxicity assay was performed 48 hrs after α-syn PFF-ACM treatment. Bars indicate mean ± S.E.M. (n=6, biologically independent primary cortical neurons). Scale bar, 20 μm. Human DA neuronal cell death was

determined by the (**c**) alamarBlue assay (n=8, biologically independent human dopaminergic neurons), (**d**) LDH assay (n=6, biologically independent human dopaminergic neurons), and (**e**) Trypan Blue live cell counting (n=6, biologically independent human dopaminergic neurons). The toxicity assay was performed 5 days after α-syn PFF-ACM treatment. Bars indicate mean ± S.E.M. (**f**, **g**) Human DA neurons were incubated with α-syn PFF-MCM or α-syn PFF-ACM with or without NLY01 (1 μM). Human DA neuronal cell death was determined by the (**f**) alamarBlue assay (n=4, biologically independent human dopaminergic neurons), and (**g**) LDH assay (n=4, biologically independent human dopaminergic neurons). Bars indicate mean ± S.E.M. Two-way ANOVA was used to test for the statistical significance, followed by Tukey's multiple comparisons test for multiple group comparisons. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. Veh-ACM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF ACM with NLY01.

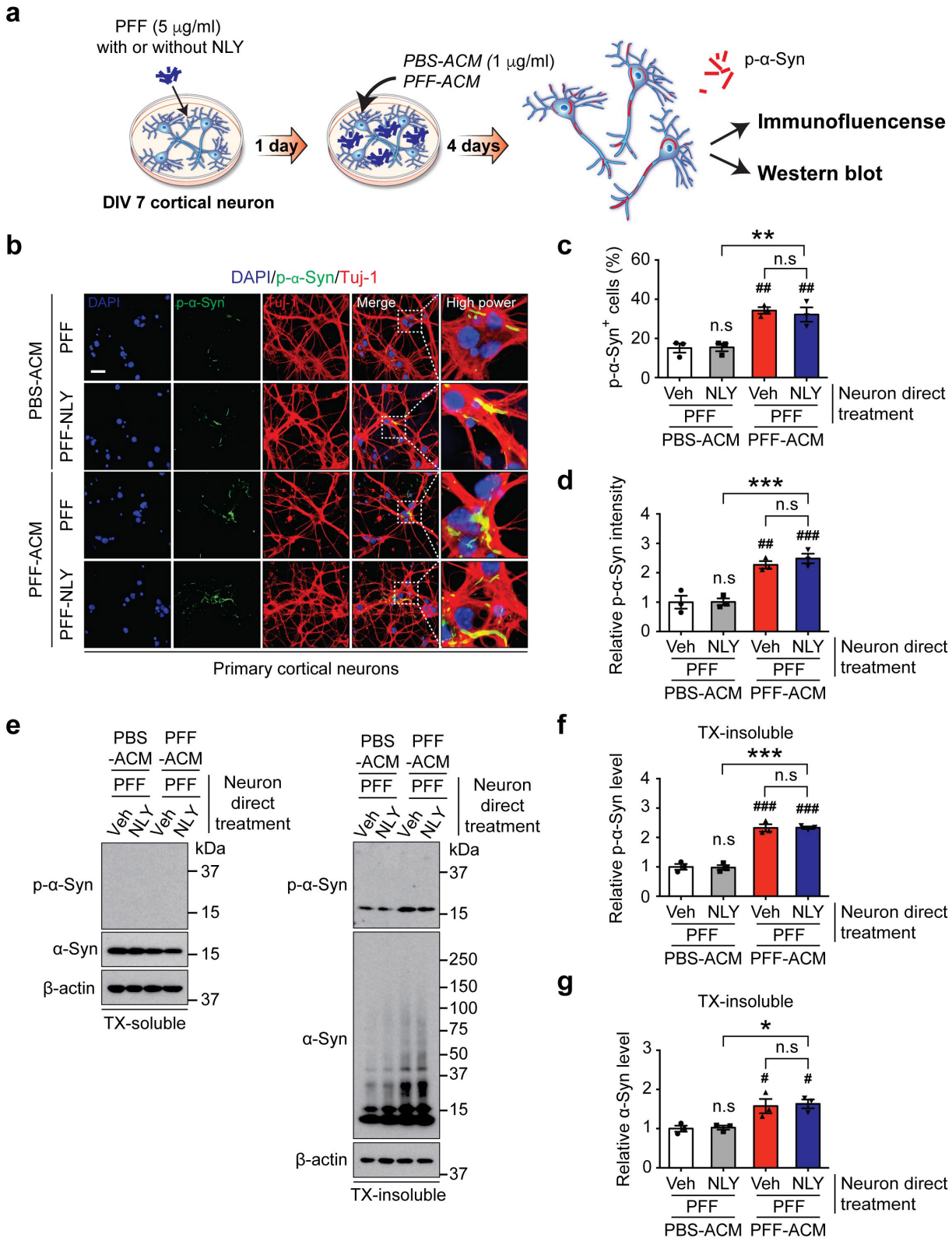
Supplementary Figure 12



Supplementary Figure 12. Inhibitory effect of NLY01 on A1 ACM-induced phosphorylation of α-syn in neurons. (**a**) Schematic diagram showing treatment of A1 ACM induced by α-syn PFF MCM into primary cortical neurons, which were pretreated with PBS or α-syn PFF for 1 day. (**b**)

Representative double-immunostaining for p-α-syn^{ser129} (green) and Tuj-1 (red) in primary cortical neurons (n=4, biologically independent primary cortical neurons). Scale bar, 10 μm. (**c**) Percentage of Tuj-1 positive neurons with p-α-syn^{ser129} (p-α-syn). Bars represent the mean ± S.E.M. (n=4, biologically independent primary cortical neurons). (**d**) Relative intensity of p-α-syn^{ser129} positive neurons. Bars represent the mean ± S.E.M. (n=4, biologically independent primary cortical neurons). (**e**) Representative immunoblots of α-syn, p-α-syn^{ser129}, Tuj-1, and β-actin in the detergent-soluble and detergent-insoluble fraction (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots). (**f, g**) Quantification of α-syn and p-α-syn^{ser12} protein levels in the detergent-insoluble (TX-100) fraction normalized to β-actin. Pre-treatment of with PFF in the murine primary cortical neurons, the expression of p-α-syn^{ser129} was further increased in the α-syn PFF ACM-treated group than in the PBS ACM treated group. Error bars represent the mean ± S.E.M. (n=3, biologically independent primary cortical neurons). Two-way ANOVA was used to test for the statistical significance, followed by Tukey's multiple comparisons test for multiple group comparisons., $^{###}P < 0.001$ vs. Veh-ACM alone; $^{*}P < 0.05$, $^{***}P < 0.001$ vs. or α-syn PFF ACM with NLY01. N.D, not detected.

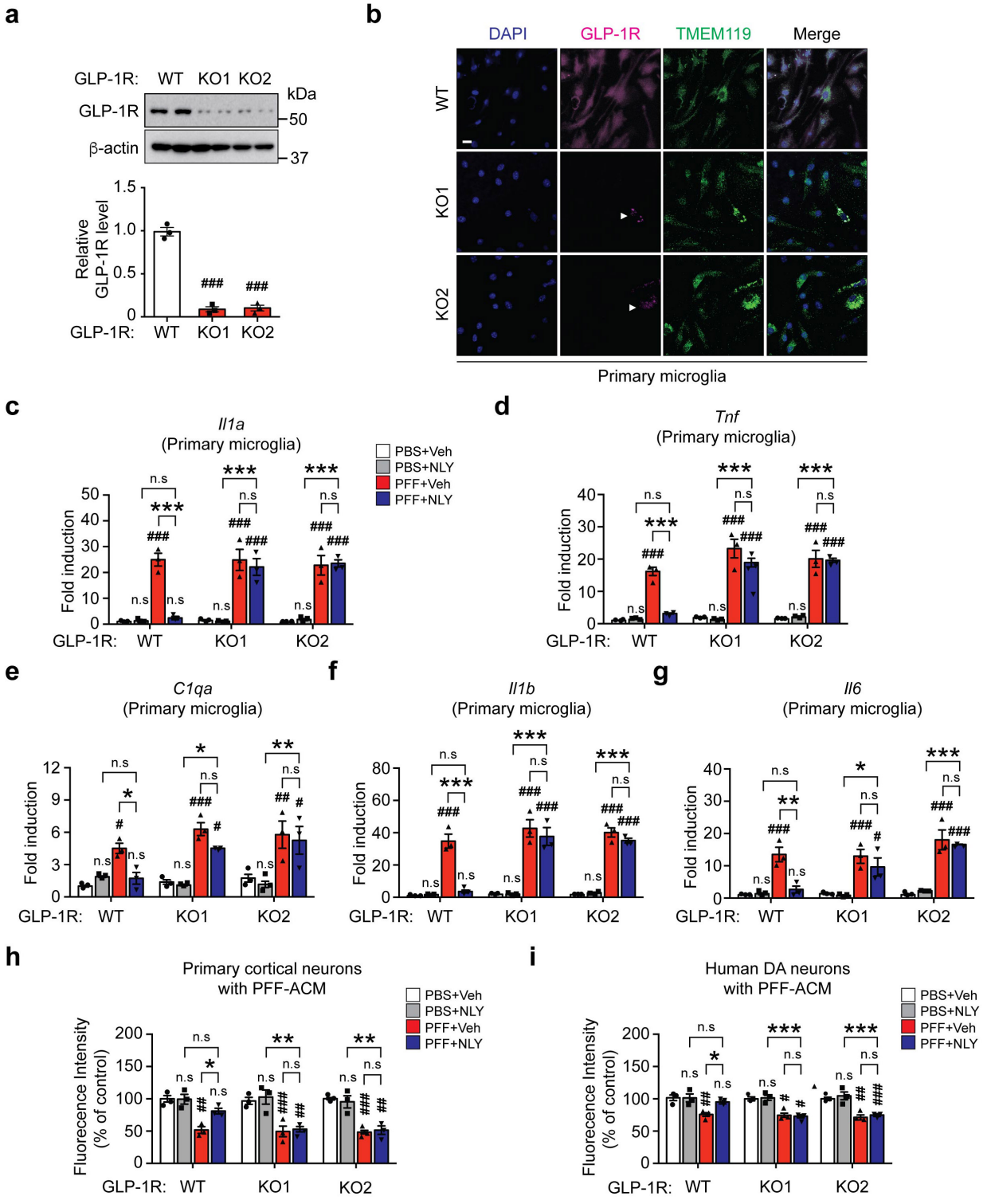
Supplementary Figure 13



Supplementary Figure 13. Direct neuronal treatment of NLY01 fails to reduce phosphorylation of α-syn in neurons. (**a**) Schematic diagram showing treatment of α-syn PFF into primary cortical neurons, which were pretreated PBS or NLY01 for 30 min. After 1 day,

primary cortical neurons were treated with PBS-ACM or PFF-ACM for 4 days. (**b**) Representative double-immunostaining for p-α-syn^{ser129} (p-α-syn) (green) and Tuj-1 (red) in primary cortical neurons (n=3, biologically independent primary cortical neurons). Scale bar, 10 μm. (**c**) Percentage of Tuj-1 positive neurons with p-α-syn^{ser129}. Bars represent the mean ± S.E.M. (n=3, biologically independent primary cortical neurons). (**d**) Relative intensity of p-α-syn^{ser129} positive neurons. Bars represent the mean ± S.E.M. (n=3, biologically independent primary cortical neurons). (**e**) Representative immunoblots of α-syn, p-α-syn^{ser129}, and β-actin in the detergent-soluble fraction, and detergent-insoluble fraction (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots). (**f-g**) Quantification of α-syn and p-α-syn^{ser129} protein levels in the detergent-insoluble (TX-100) fraction normalized to β-actin. When NLY01 was directly pre-treated with murine primary cortical neuron before α-syn ACM PFF treatment, it did not affect the expression of p-α-syn^{ser129} caused by α-syn ACM PFF. Error bars represent the mean ± S.E.M. (n=3, biologically independent primary cortical neurons). Two-way ANOVA was used to test for the statistical significance, followed by Tukey's multiple comparisons test for multiple group comparisons. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. α-syn PFF with PBS-ACM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF with NLY01 or α-syn PFF with PFF-ACM.

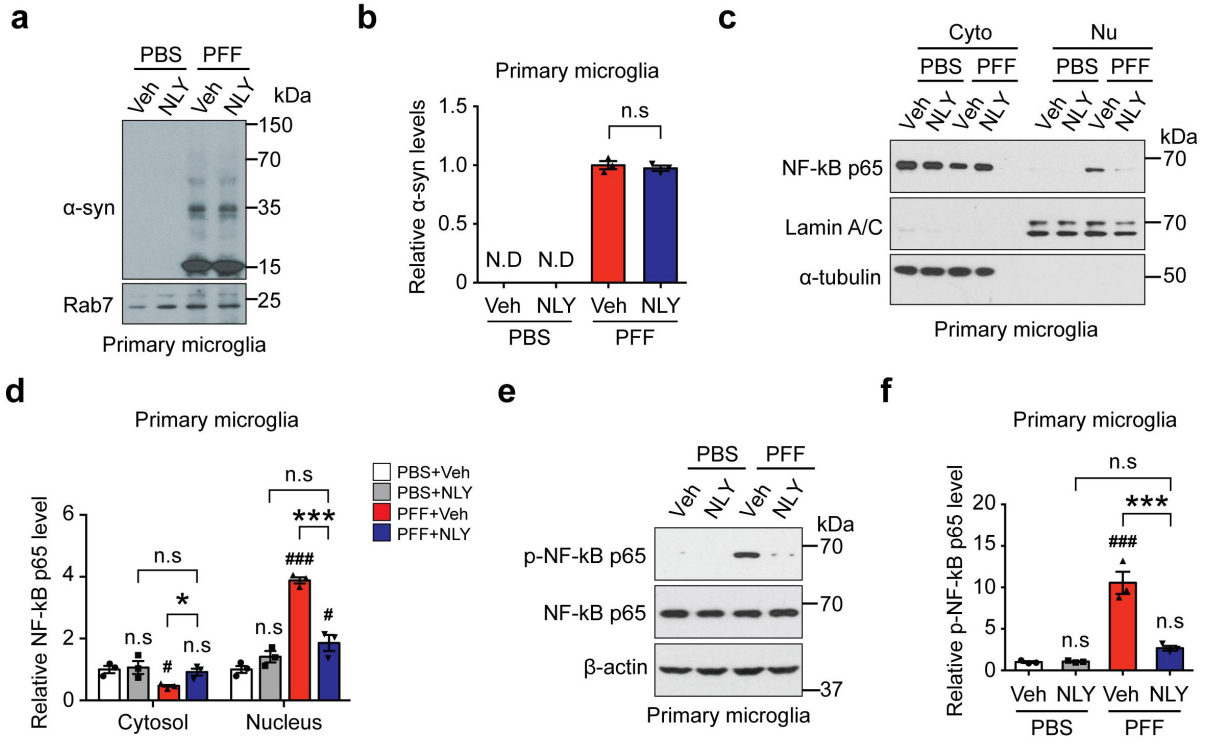
Supplementary Figure 14



Supplementary Figure 14. Protective effect of NLY01 on α-syn PFF-induced microglia activation requires GLP-1R dependent signaling. (**a**) Representative immunoblots of GLP-1R, and β-actin in the WT and lenti-CRISPR/Cas9 mediated GLP-1R KO microglia (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots). Quantification of GLP-1R

levels in the microglia normalized to β-actin. Bars represent the mean ± S.E.M. (n=3, biologically independent primary microglia, p value < 0.0001). Unpaired two-tailed Student's t test. (**b**) Lack of co-localization of TMEM119 (violet) and GLP1-R (green) in GLP-1R KO microglia. White arrow head indicates non-transduced microglia (n=4, biologically independent primary microglia). Scale bar, 10 μm. (**c-g**) Quantitative PCR analysis of NLY01 (1 μM) pretreatment on α-syn PFF-activated (1 μg/ml) WT and GLP-1R KO microglia. (**c**) *Il1a*, (**d**), *Tnfα*, (**e**) *C1qa*, (**f**) *Il1b*, and (**g**) *Il6*. Bars represent the mean ± S.E.M. (n=3, biologically independent primary microglia). (**h, i**) Mouse primary cortical neurons (48 hrs) and human DA neurons (5 days) were incubated with ACM induced by α-syn PFF treated WT or GLP-1R KO MCM with vehicle or NLY01. (**h**) Mouse cortical and (**i**) human DA neuronal death was determined by the alamarBlue assay. Bars represent the mean ± S.E.M. (n=3, biologically independent primary cortical neurons or human dopaminergic neurons). Two-way ANOVA was used to test for the statistical significance, followed by Tukey's multiple comparisons test for multiple group comparisons. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF with NLY01.

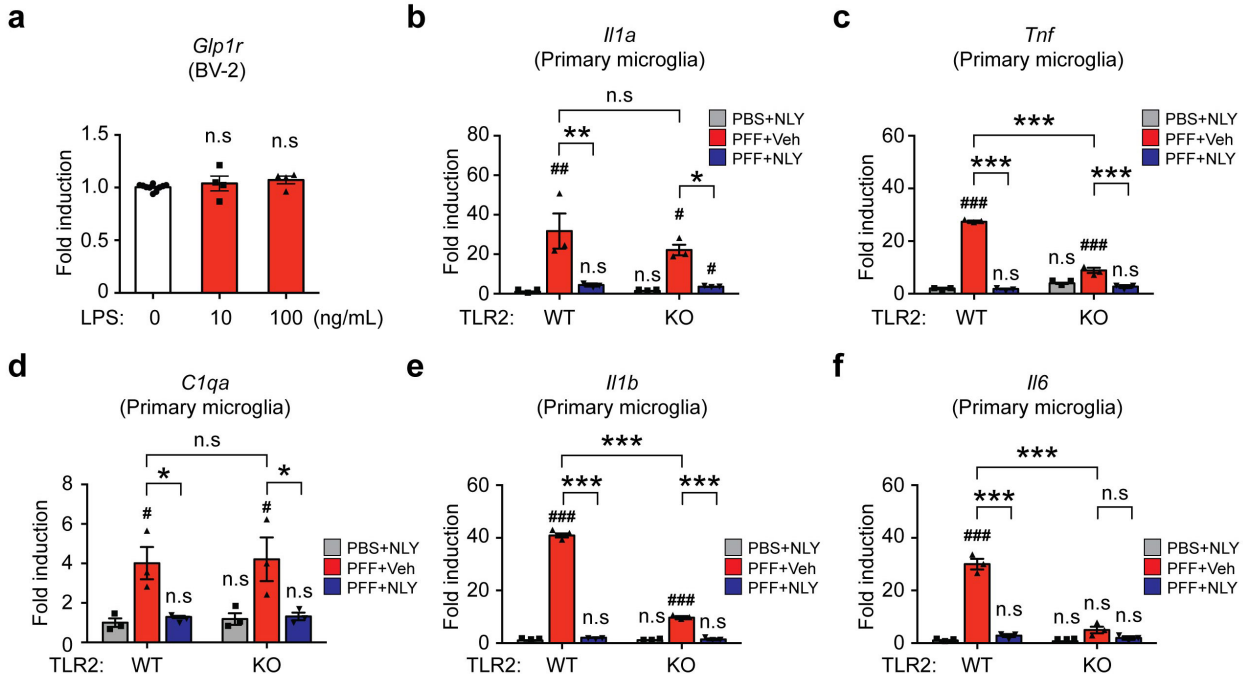
Supplementary Figure 15



Supplementary Figure 15. NLY01 inhibits α-syn PFF-induced NF-κB nuclear translocation and phosphorylation. (**a**) Representative immunoblots of internalized α-syn in primary microglia (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots). Rab7 was used as an internal control (n=3, biologically independent primary microglia). **(b**) Quantification of α-syn levels in microglia normalized to Rab7. Error bars represent the mean ± S.E.M. (n=3, biologically independent primary microglia). Unpaired two-tailed Student's t test for statistical significance. (**c**) Representative immunoblots of NF-κB p65, Lamin A/C (nucleus marker), and α-tubulin (cytosol marker) in primary microglia (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots). (**d**) Quantification of cytosolic and nuclear NF-κB p65 levels in microglia normalized to α-tubulin and Lamin A/C, respectively. Error bars represent the mean ± S.E.M. (n=3, biologically independent primary microglia). Two-way ANOVA was used to test for the statistical significance, followed by Tukey's multiple comparisons test for multiple group comparisons. (**e**) Representative immunoblots of phospho-NF-κB p65, total NF-κB p65, and β-actin in primary microglia (cropped blot images are shown, see **Supplementary Fig. 22** for full immunoblots). **(f**) Quantification of phospho-NF-κB p65 levels in the microglia normalized to NF-κB p65. Error bars represent the mean ± S.E.M. (n=3, biologically independent primary microglia). Two-way ANOVA was used to test for the statistical significance, followed by Tukey's multiple

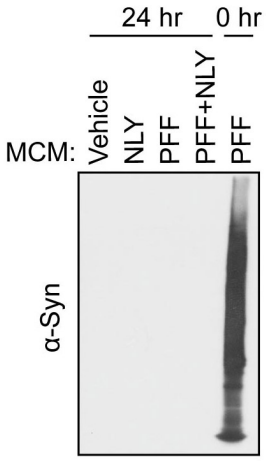
comparisons test for multiple group comparisons. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. PBS with vehicle; $^{*}P < 0.05$, $^{***}P < 0.001$ vs. or α-syn PFF with NLY01.

Supplementary Figure 16



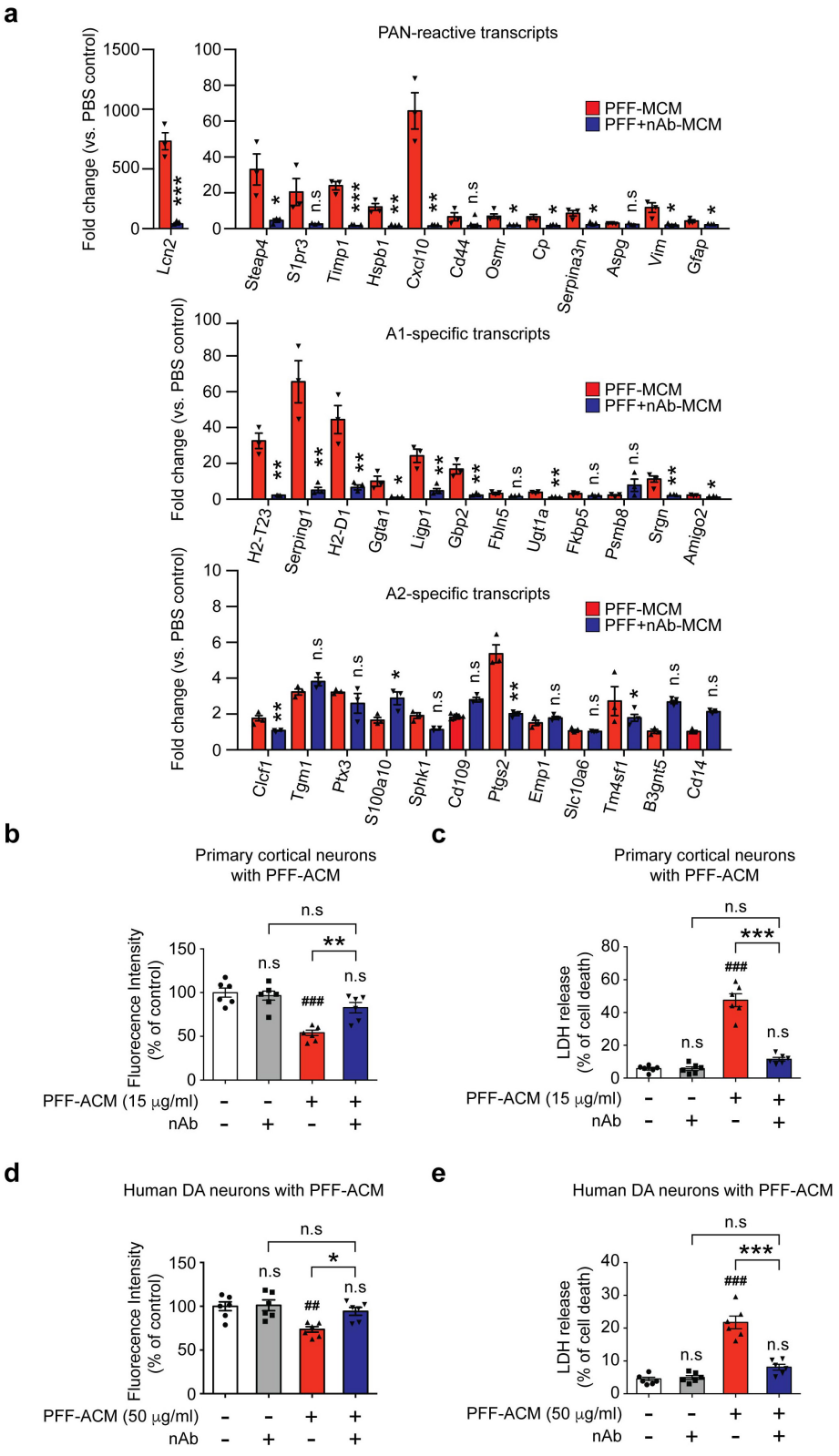
Supplementary Figure 16. Protective effect of NLY01 on microglial activation is not dependent on TLR2. (**a**) BV2 cells were treated with LPS for 4hrs. The *Glp1r* mRNA expression was analyzed by real-time RT-PCR. Bars represent the mean ± S.E.M. (Control n=10, LPS n=4, biologically independent BV-2 cell lines). (**b-f**) Quantitative PCR analysis of NLY01 (1 μM) pretreatment on α-syn PFF-induced (1 μg/ml) microglial activation markers in WT microglia and Toll like receptor (TLR)-2 KO microglia. (**b**) *Il1a*, (**c**), *Tnfα*, (**d**) *C1qa*, (**e**) *Il1b*, and (**f**) *Il6*. Bars represent the mean ± S.E.M. (n=3, biologically independent primary microglia). Two-way ANOVA was used for statistical analysis followed by Tukey's multiple comparisons test for multiple group comparisons. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS with NLY01; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. or α-syn PFF with NLY. n.s, not significant.

Supplementary Figure 17



Supplementary Figure 17. No remaining α-syn PFF in the MCM. 24 hrs after treatment of α-syn PFF with or without NLY01 (1 μM) the levels of α-syn PFF in the MCM were determined by western blot analysis using an α-syn antibody (n=3, biologically independent primary microglia conditional media). The α-syn PFF-treated MCM at 0 hr was used as a positive control.

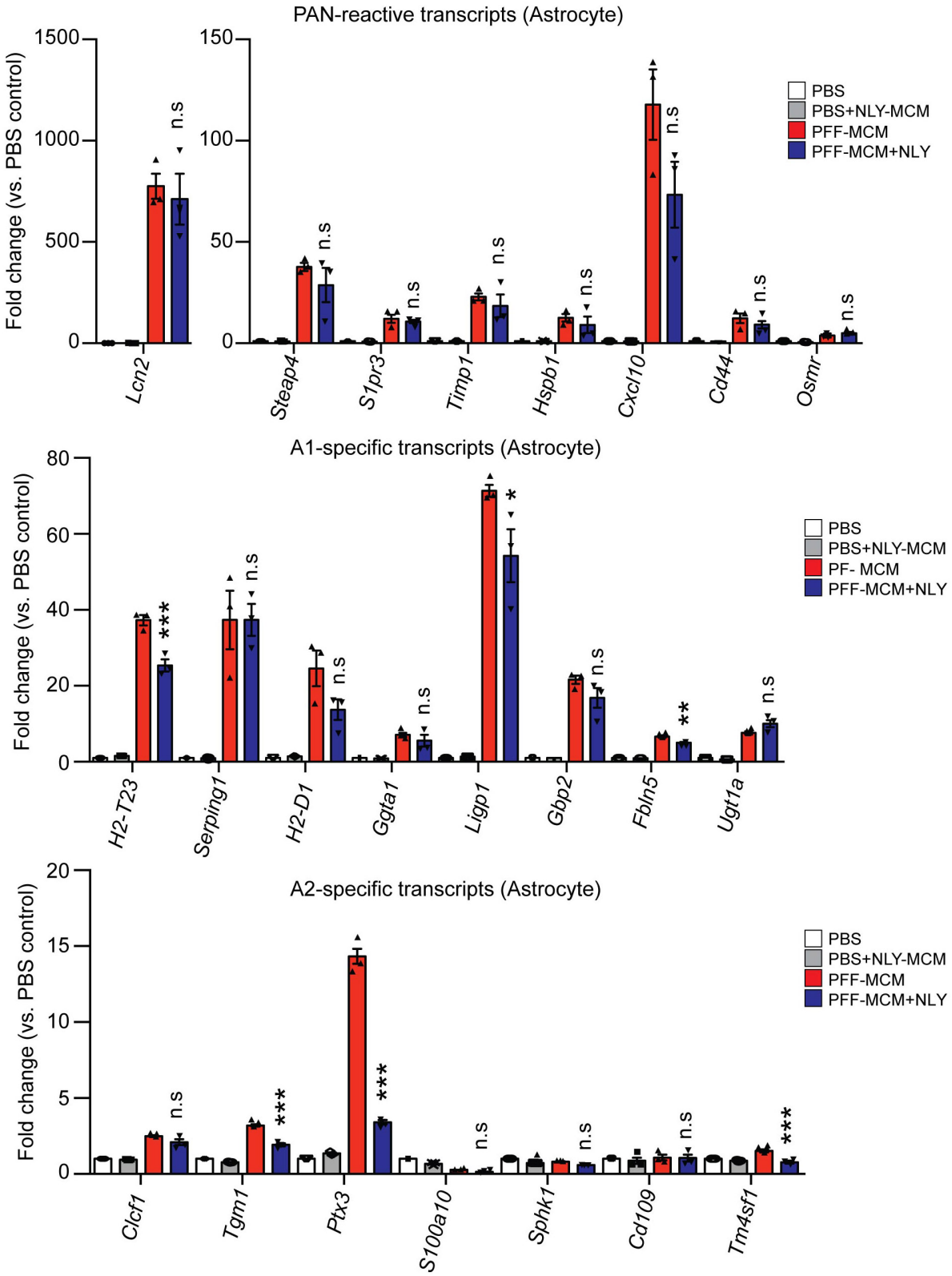
Supplementary Figure 18



Supplementary Figure 18. A1 neutralizing antibodies inhibit α-syn PFF ACM-induced neuronal death. (**a**) Purified primary astrocytes were activated by α-syn PFF MCM pre-treated

with IgG or neutralizing antibodies (nAb) to IL-1α, TNFα, and C1q for 24 hrs. Formation of A1 astrocytes was determined using quantitative PCR analysis. Bars represent the mean ± S.E.M. (n=3, biologically independent primary astrocytes). Unpaired two-tailed Student's t test for statistical significance. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. or α-syn PFF MCM. (**b, c**) Mouse cortical neurons were incubated with ACM activated by α-syn PFF MCM pre-treated with or without nAb (10 μg/ml of IL-1α, TNFα, and C1q) for 48 hrs. Mouse cortical neuronal cell death was determined by the (**b**) alamarBlue assay, and (**c**) LDH assay. Bars represent the mean ± S.E.M. (n=6, biologically independent primary cortical neurons). (**d, e**) DIV 60 human DA neurons were incubated with ACM activated by MCM pre-treated with or without nAb (10 μg/ml of IL-1α, TNFα, and C1q) for 5 days. Human DA neuronal cell death was determined by the (**d**) alamarBlue assay, and (**e**) LDH assay. Bars represent the mean ± S.E.M. (n=6, biologically independent human dopaminergic neurons). Two-way ANOVA was used to test for the statistical significance, followed by Tukey's multiple comparisons test for multiple group comparisons. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. Veh-ACM with IgG control; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. or α-syn PFF ACM with nAb.

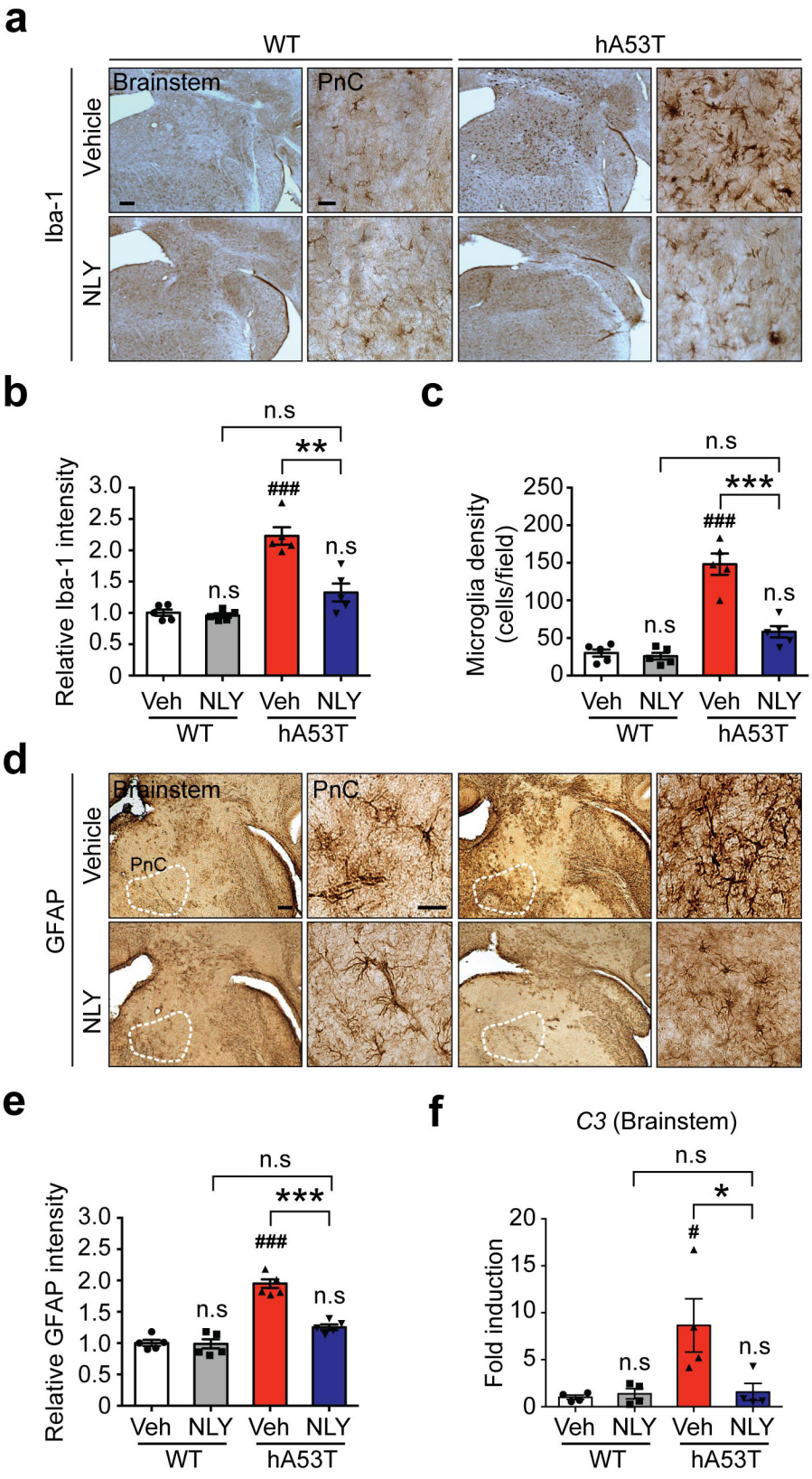
Supplementary Figure 19



Supplementary Figure 19. Astrocytes treatment with NLY01 does not prevent the formation of A1 astrocytes by α-syn PFF-activated MCM. Purified primary astrocytes were pretreated with NLY01 (1 μM), activated by α-syn PFF-activated MCM for 24 hrs, and then subjected to

quantitative PCR analysis. Bars represent the mean ± S.E.M. (n=3, biologically independent primary astrocytes). Two-way ANOVA was used for statistical analysis followed by Tukey's multiple comparisons test for multiple group comparisons. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. or α-syn PFF MCM. n.s, not significant. NLY, NLY01; MCM, microglial conditioned media.

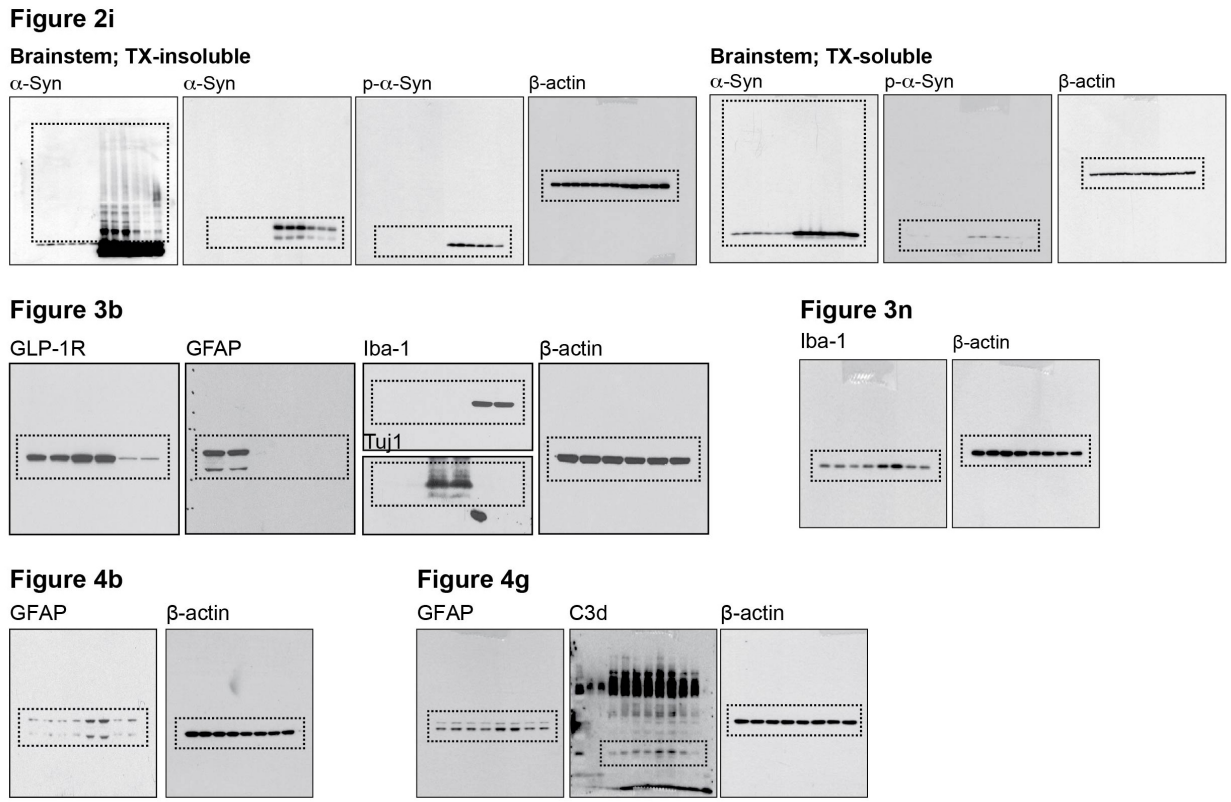
Supplementary Figure 20



Supplementary Figure 20. Inhibition of microglial and astrocyte activation by NLY01 in hA53T α-synuclein transgenic mice. (**a**) Representative immunohistochemical images of Iba-1

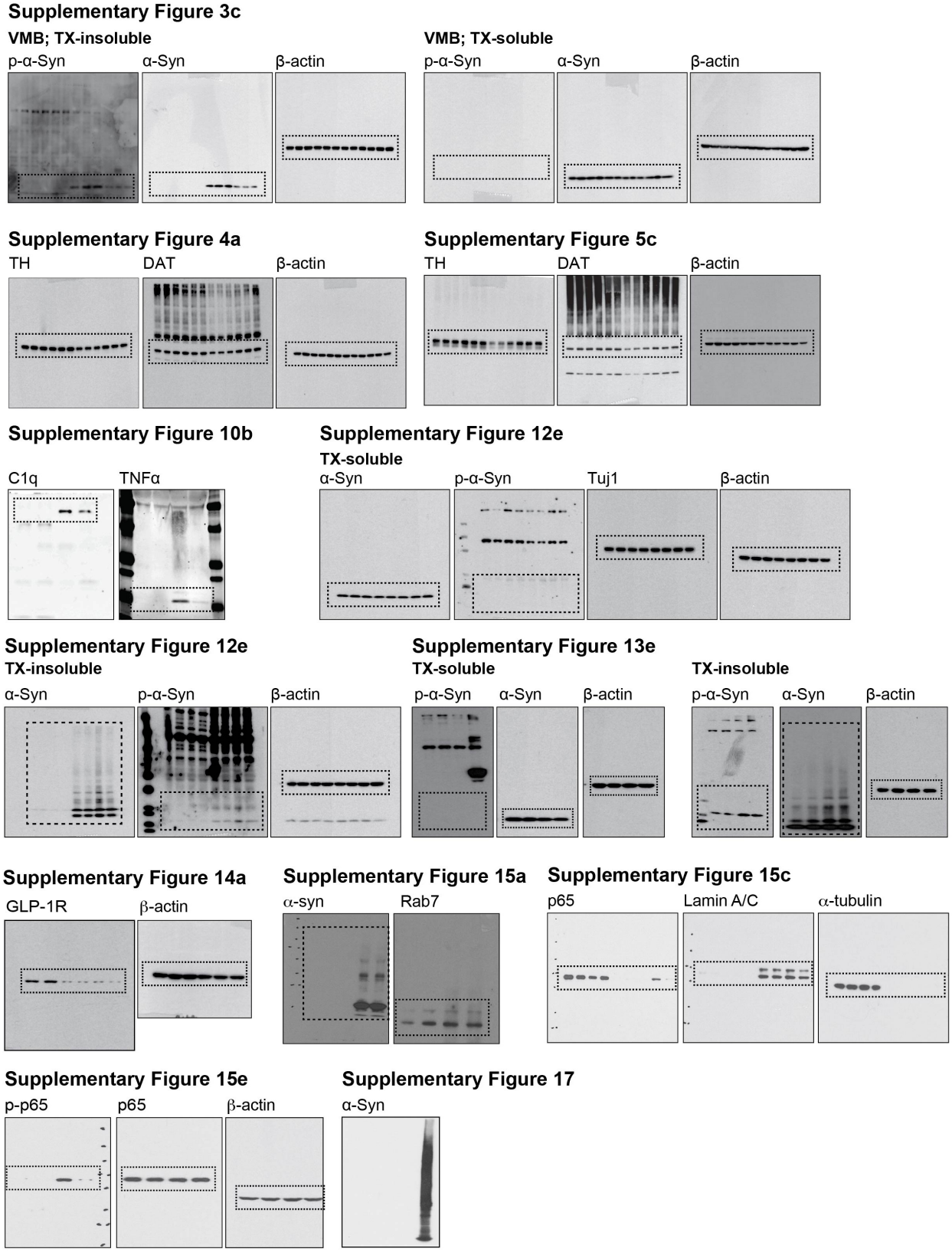
in the brainstem. Scale bar, 200 μm or 50 μm. (n=5, biologically independent animals). **(b-c)** Quantification of Iba-1 positive cells in the brainstem of WT and hA53T α-syn Tg mice treated with vehicle or NLY01. Error bars represent the mean S.E.M. (n=5, biologically independent animals). (**d**) Representative immunohistochemical images of GFAP in the brainstem. (n=5, biologically independent animals). Scale bar, low power 200 μm, high power 50 μm. (**e**) Quantification of GFAP in the brainstem of WT and hA53T α-syn Tg mice treated with vehicle or NLY01. Error bars represent the mean S.E.M. (n=5, biologically independent animals). (**f**) Increase in the expression of C3 transcript, which was prevented by NLY01 treatment in hA53T α-syn Tg mice. Bars represent the mean ± S.E.M. (n=4, biologically independent animals). Two-way ANOVA was used to test for the statistical significance, followed by Tukey's multiple comparisons test for multiple group comparisons. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. WT with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. or hA53T α-syn Tg with NLY01. PnC, Pontine reticular nucleus, caudal part.

Supplementary Figure 21



Supplementary Figure 21. Original full western blot image of main manuscript.

Supplementary Figure 22



Supplementary Figure 22. Original full western blot image of supplementary figures.

Supplementary Table 1.

	Dose (μg/kg)	C_{max} (ng/ml)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{inf} (ng·h/ml)	MRT(h)
[Cys^{40}] Exendin-4	2.5	31.3 ± 8.2	0.8 ± 0.4	2.7 ± 0.9	40.3 ± 10.5	2.5 ± 0.3
NLY01	32	52.7 ± 18.6	72 ± 33.9	88.0 ± 10.9	6833.5 ± 13.7	114.0 ± 17.5

Supplementary Table 1. Pharmacokinetic parameters of [Cys^{40}] Exendin-4 and NLY01 in monkeys. C_{max}, maximum observed plasma concentration; T_{max}, time of maximal observed plasma concentration; $t_{1/2}$, elimination half-life; AUC_{inf}, are under the plasma concentration curve from zero to infinity; MRT, mean residence time. (n=2).

Supplementary Table 2.

Case #	SEX	AGE	Race	PMD (Hr)	FDX	FRZ
107	M	71	W	14	Control	SN
123	F	80	W	66	Control	SN
155	M	72	W	24	Control	SN
289	F	68	W	35	Control	SN
384	M	68	W	14	Control	SN
507	F	87	W	23	Control	SN
705	M	73	W	9	Control	SN
710	M	62	W	14	Control	SN
2052	M	79	W	16	Control	SN
2193	M	89	W	9	Control	SN
2450	M	79	W	21	PD with dementia, neurofib degen	SN
2461	M	76	W	29	PD with dementia, AD possible	SN
2467	M	72	W	15	PD	SN
2489	M	86	W	19	Lewy body disease, incipient AD	SN
2490	M	90	W	7	PD, TAU BRAAK4, TBI possible	SN
2526	F	88	W	6	PD with dementia, trauma, mixed dementia (AD+PD)	SN
2536	M	65	W	6	PD, cerebrovascular disease (NC)	SN
2541	M	89	W	24	PD, AD possible	SN
2544	M	89	W	16	PD with dementia	SN
2545	F	95	W	14	PD, neurofib degen, cerebrovascular disease (NC)	SN

Supplementary Table 2. Human post-mortem tissues of Parkinson's disease.

Supplementary Table 3.

mRNA		PBS		PFF		ANOVA	
		PBS (1)	NLY01 (2)	PBS (3)	NLY01 (4)	(1) & (3)	(3) & (4)
PAN	*Lcn2*	1	1.16 ± 0.31	1334.54 ± 128.64	503.26± 179.50	###	**
	Steap4	1	1.35 ± 0.43	74.42 ± 22.60	32.67 ± 22.11	#	n.s.
	S1pr3	1	1.02 ± 0.14	17.02 ± 3.74	3.71± 1.52	##	**
	Timp1	1	1.42 ± 0.16	24.55 ± 5.15	8.71± 4.00	##	*
	Hspb1	1	1.13 ± 0.13	8.54 ± 2.02	2.54 ± 1.07	##	*
	Cxcl10	1	1.19 ± 0.36	74.12 ± 4.86	27.65 ± 12.49	###	**
	Cd44	1	0.71 ± 0.28	8.13 ± 3.53	3.68 ± 1.39	n.s.	n.s.
	Osmr	1	0.68 ± 0.24	7.91 ± 0.54	5.23 ± 2.25	#	n.s.
	Cp	1	0.62 ± 0.23	3.30 ± 0.11	1.81 ± 0.63	##	n.s.
	Serpinga3n	1	0.67 ± 0.24	5.48 ± 0.20	2.77 ± 0.94	###	*
	Aspg	1	0.86 ± 0.34	2.13 ± 0.09	1.33 ± 0.34	#	n.s.
	Vim	1	0.72 ± 0.24	8.38 ± 2.68	1.23 ± 0.36	#	*
	Gfap	1	0.65 ± 0.22	1.00 ± 0.03	0.76 ± 0.15	n.s.	n.s.
A1	*H2-T23*	1	1.40 ± 0.93	24.82 ± 1.35	8.46 ± 2.13	###	***
	Serping1	1	0.99 ± 0.56	64.43 ± 22.99	5.89 ± 2.11	#	*
	H2-D1	1	1.11 ± 0.65	21.91 ± 4.90	5.23 ± 1.57	##	**
	Ggta1	1	1.41 ± 0.86	8.12 ± 0.82	2.44 ± 0.24	###	***
	Ligp1	1	1.53 ± 0.82	45.44 ± 10.28	12.65 ± 4.38	##	*
	Gbp2	1	1.01 ± 0.60	9.07 ± 0.09	3.06 ± 0.84	###	***
	Fbln5	1	0.77 ± 0.38	0.95 ± 0.13	0.83 ± 0.11	n.s.	n.s.
	Ugt1a	1	1.89 ± 0.87	4.03 ± 0.80	1.43 ± 0.40	#	n.s.
	Fkbp5	1	1.07 ± 0.53	1.10 ± 0.19	0.82 ± 0.03	n.s.	n.s.
	Psmb8	1	1.40 ± 0.84	11.95 ± 2.44	4.66 ± 1.29	##	*
	Srgn	1	1.70 ± 1.05	9.64 ± 1.89	3.63 ± 0.90	##	*
	Amigo2	1	0.95 ± 0.34	3.55 ± 0.05	1.83 ± 0.71	##	n.s.
A2	*Clcf1*	1	0.72 ± 0.26	1.83 ± 0.13	1.33 ± 0.41	n.s.	n.s.
	Tgm1	1	0.69 ± 0.22	4.49 ± 0.23	2.67 ± 0.98	##	n.s.
	Ptx3	1	0.70 ± 0.30	11.16 ± 1.41	9.17 ± 4.10	#	n.s.
	S100a10	1	0.62 ± 0.20	0.59 ± 0.03	0.60 ± 0.11	n.s.	n.s.
	Sphk1	1	0.79 ± 0.28	2.82 ± 0.11	1.86 ± 0.64	#	n.s.
	Cd109	1	0.61 ± 0.22	1.06 ± 0.02	0.84 ± 0.20	n.s.	n.s.
	Ptgs2	1	0.81 ± 0.28	8.89 ± 3.48	6.90 ± 2.91	n.s.	n.s.
	Emp1	1	0.95 ± 0.35	1.38 ± 0.06	1.21 ± 0.34	n.s.	n.s.
	Slc10a6	1	1.77 ± 0.70	3.64 ± 0.12	2.00 ± 0.66	#	n.s.
	Tm4sf1	1	0.75 ± 0.26	1.79 ± 0.06	1.22 ± 0.36	n.s.	n.s.
	B3gnt5	1	0.92 ± 0.24	2.59 ± 1.44	2.04 ± 1.27	n.s.	n.s.
	Cd14	1	0.95 ± 0.46	5.30 ± 2.22	1.94 ± 0.87	n.s.	n.s.

Supplementary Table 3. α-syn PFF MCM preferentially induces markers of A1 astrocytes, while not perturbing A2 specific transcripts. Center values represent the mean ± S.E.M. (n=3, biologically independent primary astrocytes). Two-way ANOVA followed by Tukey's multiple comparisons test was used to test for statistical significance. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF with Veh. n.s, not significant.

Supplementary Table 4.

mRNA		PBS		PFF		ANOVA	
		PBS (1)	NLY01 (2)	PBS (3)	NLY01 (4)	(1) & (3)	(3) & (4)
PAN	*Lcn2*	1	1.03 ± 0.35	14.41 ± 5.41	2.96 ± 0.92	#	n.s.
	Steap4	1	1.19 ± 0.75	9.12 ± 2.99	2.04 ± 0.85	#	*
	S1pr3	1	1.1.42 ± 0.47	13.84 ± 1.26	6.55 ± 2.06	###	**
	Timp1	1	0.58 ± 0.09	22.05 ± 6.44	4.11 ± 2.23	##	*
	Hspb1	1	0.76 ± 0.22	12.92 ± 6.86	1.63 ± 0.39	n.s.	n.s.
	Osmr	1	1.49 ± 0.54	19.79 ± 4.95	2.61 ± 0.86	##	**
A1	*H2-T23*	1	0.72 ± 0.33	9.60 ± 3.71	1.27 ± 0.46	#	*
	Serping1	1	0.82 ± 0.39	7.45 ± 4.06	0.61 ± 0.14	n.s.	n.s.
	H2-D1	1	0.83 ± 0.16	12.30 ± 3.24	1.67 ± 0.39	##	**
	Ggta1	1	1.19 ± 0.52	14.23 ± 5.29	2.76 ± 0.44	#	*
	Ligp1	1	0.85 ± 0.31	11.32 ± 4.98	1.62 ± 0.36	n.s.	n.s.
	Gbp2	1	1.13 ± 0.70	10.64 ± 2.43	1.81 ± 0.59	##	**
	Fbln5	1	0.73 ± 0.23	11.48 ± 5.92	1.97 ± 0.48	n.s.	n.s.
	Ugt1a	1	0.94 ± 0.54	8.25 ± 1.87	1.77 ± 0.46	##	**
A2	*Clcf1*	1	0.43 ± 0.14	3.26 ± 0.64	1.40 ± 0.51	n.s.	n.s.
	Tgm1	1	1.02 ± 0.91	1.89 ± 1.06	0.26 ± 0.01	n.s.	n.s.
	Ptx3	1	0.40 ± 0.13	3.64 ± 0.85	1.66 ± 0.60	n.s.	n.s.
	S100a10	1	0.66 ± 0.03	5.54 ± 1.53	2.01 ± 0.35	n.s.	n.s.
	Sphk1	1	0.55 ± 0.13	3.30 ± 1.36	1.37 ± 0.33	n.s.	n.s.
	Cd109	1	0.43 ± 0.22	3.79 ± 1.19	2.73 ± 0.86	n.s.	n.s.
	Tm4sf1	1	0.54 ± 0.07	3.92 ± 1.28	2.79 ± 1.34	n.s.	n.s.
	B3gnt5	1	0.44 ± 0.13	3.26 ± 0.63	1.63 ± 0.59	n.s.	n.s.

Supplementary Table 4. Intrastriatal injection of α-syn PFF primarily induces A1 astrocyte specific transcripts that are blocked by NLY01. Center values represent the mean ± S.E.M. (n=4, biologically independent primary astrocytes). Two-way ANOVA followed by Tukey's multiple comparisons test was used to test for statistical significance. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF with NLY01 or with Veh. n.s, not significant.

Supplementary Table 5.

Antibodies	Source/Cat. No.	Host	Dilution
α-Synuclein	BD Bioscience (610787)	Mouse	1:2,000 (WB)
p-α-synuclein^{Ser129}	Biolegend (825701) Abcam (ab168381)	Mouse Rabbit	1:1,000 (IHC, IF) 1:500 (WB)
Tyrosine Hydroxylase (TH)	Novus Biologicals (NB300-109)	Rabbit	1:2,000 (WB) 1:1,000 (IHC, IF)
Dopamine transporter (DAT)	Sigma (D6944)	Rabbit	1:1,000 (WB)
Tuj1	Biolegend (802001)	Rabbit	1:2,000 (WB) 1:1,000 (IF)
C3d	R&D system (AF2655)	Goat	1:50 (IF) 1:500 (WB)
C31	Abcam (ab24590)	Mouse	1:100 (IF)
GFAP	Dako (Z033429) EMD Millipore (MAB360)	Rabbit Mouse	1:500 (IHC, IF) 1:1,000 (WB)
Iba-1	Wako (019-19741) Wako (016-20001)	Rabbit Rabbit	1:500 (IHC) 1:1,000 (WB)
GLP-1R	Santa Cruz (SC-390774)	Mouse	1:2,000 (WB)
TNFα	Cell signaling (3707) Cell signaling (7321)	Rabbit Rabbit	1:1000 (WB) 10 μg/ml (neutralizing)
C1q	Abcam (ab71089) Quidel (A301)	Mouse Goat	1:1000 (WB) 10 μg/ml (neutralizing)
TMEM119	Abcam (ab209064)	Rabbit	1:1000 (IF)
β-actin-HRP	Sigma-Aldrich (A3854)		1:50,000 (WB)
IL-1α	Abcam (ab9614)	Rabbit	10 μg/ml (neutralizing)
IgG control	Abcam (ab27472)	Rabbit	10 μg/ml (neutralizing)
NF-κB P65	Cell signaling (8242S)	Rabbit	1:1000 (WB)
p-NF-κB p65	Cell signaling (3033S)	Rabbit	1:1000 (WB)
Lamin A/C	Santa Cruz (SC-7293)	Rabbit	1:1000 (WB)
α-tubulin	Cell signaling (2144)	Rabbit	1:2000 (WB)

Supplementary Table 5. Antibodies used in this study.

Supplementary Table 6.

Genes	Forward primer	Reverse primer	Size (bp)
Lcn2	CCAGTTCGCCATGGTATTTT	CACACTCACCACCCATTCAG	206
Steap4	CCCGAATCGTGTCTTTCCTA	GGCCTGAGTAATGGTTGCAT	262
S1pr3	AAGCCTAGCGGGAGAGAAAC	TCAGGGAACAATTGGGAGAG	197
Timp1	AGTGATTTCCCCGCCAACTC	GGGGCCATCATGGTATCTGC	123
Hspb1	GACATGAGCAGTCGGATTGA	GGATGGGGTGTAGGGGTACT	265
Cxcl10	CCCACGTGTTGAGATCATTG	CACTGGGTAAAGGGGAGTGA	211
Cd44	ACCTTGGCCACCACTCCTAA	GCAGTAGGCTGAAGGGTTGT	299
Osmr	GTGAAGGACCCAAAGCATGT	GCCTAATACCTGGTGCGTGT	199
Cp	TGTGATGGGAATGGGCAATGA	AGTGTATAGAGGATGTTCCAGGTCA	282
Serpinga3n	CCTGGAGGATGTCCTTTCAA	TTATCAGGAAAGGCCGATTG	233
Aspg	GCTGCTGGCCATTTACACTG	GTGGGCCTGTGCATACTCTT	133
Vim	AGACCAGAGATGGACAGGTGA	TTGCGCTCCTGAAAAACTGC	169
Gfap	AGAAAGGTTGAATCGCTGGA	CGGCGATAGTCGTTAGCTTC	299
H2-T23	GGACCGCGAATGACATAGC	GCACCTCAGGGTGACTTCAT	212
Serping1	ACAGCCCCCTCTGAATTCTT	GGATGCTCTCCAAGTTGCTC	299
H2-D1	TCCGAGATTGTAAAGCGTGAAGA	ACAGGGCAGTGCAGGGATAG	204
Ggta1	GTGAACAGCATGAGGGGTTT	GTTTTGTTGCCTCTGGGTGT	115
Ligp1	GGGGCAATAGCTCATTGGTA	ACCTCGAAGACATCCCCTTT	104
Gbp2	GGGGTCACTGTCTGACCACT	GGGAAACCTGGGATGAGATT	285
Fbln5	CTTCAGATGCAAGCAACAA	AGGCAGTGTCAGAGGCCTTA	281
Ugt1a	CCTATGGGTCACTTGCCACT	AAAACCATGTTGGGCATGAT	136
Fkbp5	TATGCTTATGGCTCGGCTGG	CAGCCTTCCAGGTGGACTTT	194
Psmb8	CAGTCCTGAAGAGGCCTACG	CACTTTCACCCAACCGTCTT	121
Srgn	GCAAGGTTATCCTGCTCGGA	TGGGAGGGCCGATGTTATTG	134
Amigo2	GAGGCGACCATAATGTCGTT	GCATCCAACAGTCCGATTCT	263
Clcf1	CTTCAATCCTCCTCGACTGG	TACGTCGGAGTTCAGCTGTG	176
Tgm1	CTGTTGGTCCCGTCCCAAA	GGACCTTCCATTGTGCCTGG	97
Ptx3	AACAAGCTCTGTTGCCCATT	TCCCAAATGGAACATTGGAT	147
S100a10	CCTCTGGCTGTGGACAAAAT	CTGCTCACAAGAAGCAGTGG	238
Sphk1	GATGCATGAGGTGGTGAATG	TGCTCGTACCCAGCATAGTG	135
Cd109	CACAGTCGGGAGCCCTAAAG	GCAGCGATTTCGATGTCCAC	147
Ptgs2	GCTGTACAAGCAGTGGCAAA	CCCCAAAGATAGCATCTGGA	232
Emp1	GAGACACTGGCCAGAAAAGC	TAAAAGGCAAGGGAATGCAC	183
Slc10a6	GCTTCGGTGGTATGATGCTT	CCACAGGCTTTTCTGGTGAT	217
Tm4sf1	GCCCAAGCATATTGTGGAGT	AGGGTAGGATGTGGCACAAG	258
B3gnt5	CGTGGGGCAATGAGAACTAT	CCCAGCTGAACTGAAGAAGG	207
Cd14	GGACTGATCTCAGCCCTCTG	GCTTCAGCCCAGTGAAAGAC	232
Tnfα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG	61
Il1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT	89
Il1a	GCACCTTACACCTACCAGAGT	AAACTTCTGCCTGACGAGCTT	63
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC	76

C1q	TCTGCACTGTACCCGGCTA	CCCTGGTAAATGTGACCCTTTT	232
C3	CCAGCTCCCCATTAGCTCTG	GCACTTGCCTCTTTAGGAAGTC	159
Glp1r	ACGGTGTCCCTCTCAGAGAC	ATCAAAGGTCCGGTTGCAGAA	117

Supplementary Table 6. Primers used in this study.